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Review

Thicker Than Blood: Conserved Mechanisms in *Drosophila* and Vertebrate Hematopoiesis

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Blood development in *Drosophila melanogaster* shares several interesting features with hematopoiesis in vertebrates, including spatiotemporal regulation as well as the use of similar transcriptional regulators and signaling pathways. In this review, we describe what is known about hematopoietic development in *Drosophila* and the various cell types generated and their functions. Additionally, the molecular genetic mechanisms of hematopoietic cell fate determination and commitment within *Drosophila* blood cell lineages are discussed and compared to vertebrate mechanisms.

Similarities between hematopoietic development in *Drosophila melanogaster* and vertebrates have recently been demonstrated by several investigations illuminating the conserved function of signaling pathways and transcription factors regulating proliferation, differentiation, and lineage commitment. The repertoire of *Drosophila* blood cell types and functions are less varied than in vertebrates, but several key components regulating hematopoietic development have been conserved through roughly 550 million years of evolution. Because of these relationships, *Drosophila* has been and continues to be a powerful system to describe basic molecular genetic mechanisms regulating these processes.

Vertebrate blood consists of multiple cell types that perform varied and specific functions. Though distinct, all blood cell types are derived from a common, pluripotent precursor or hematopoietic stem cell (Kondo et al., 2003). *Drosophila* blood consists of only a few terminally differentiated types whose functions resemble those of the cells of the vertebrate myeloid lineage. Although no hematopoietic stem cells have been identified, *Drosophila* blood cells are thought to be derived from a common set of hematopoietic precursors.

The onset of vertebrate hematopoietic development, generally termed primitive hematopoiesis, occurs during embryogenesis when the extraembryonic yolk sac (or an equivalent site) gives rise to blood precursors, which are primarily erythroid in nature (Godin and Cumano, 2002). A second round of de novo hematopoietic development, termed definitive hematopoiesis, occurs in the mesodermal aorta/gonad/mesonephros (AGM) region of the embryo proper and gives rise to cells that will seed subsequent hematopoietic sites, such as the fetal liver and the bone marrow in mammals, as well as to all blood cell types found in the mature organism (Dzierzak, 1999). The myeloid lineage gives rise to multiple cell types including erythrocytes, megakaryocytes (from which platelets are derived), and macrophages, which primarily function as professional phagocytes in the context of both development and innate immunity (Akashi et al., 2000). The lymphoid lineage primarily mediates adaptive immunity through the production of B and T cells and natural killer cells (Kondo et al., 1997).

Before we continue with a description of *Drosophila* hematopoiesis, a short description of its development is warranted (for a review, see Bate and Martinez Arias, 1993). First there is an embryonic phase, which has been divided into 17 distinct stages, that establishes the basic structures of the subsequent larval stage (Campos-Ortega and Hartenstein, 1997). Larvae are developmentally chimeric in that they contain tissues specific to the larval stage as well as separate cell populations, collectively termed imaginal discs, which will eventually give rise to the adult fly. Essentially, the larval stage, which is divided into three successive intervals or instars (first, second, and third), represents a period of growth and maturation of the imaginal discs. At the end of the third larval instar, pupariation ensues—the larval cuticle hardens and metamorphosis begins, during which obsolete larval tissues histolyze and are removed while the imaginal discs further develop into the adult form. At the end of metamorphosis, the adult fly emerges from the pupal case and begins searching for food and mate.

As in vertebrates, hematopoiesis in *Drosophila* can be described as a biphasic developmental process that serves to populate the embryo, larva, and adult with mature blood cells (Lebestky et al., 2000). *Drosophila* blood cells, or hemocytes, have at least two significant roles in development and immunity. Developmentally, hemocytes seek out and remove dead cells and debris as well as secrete and remodel extracellular matrix (ECM) components critical to morphogenesis (Fessler et al., 1994; Murray et al., 1995; Franc et al., 1996). Hemocytes also mediate innate, humoral immune responses, where they act as sentinels by monitoring the environment for pathogens and signaling to the larval fat body, which is the major source of antimicrobial peptide production (Tzou et al., 2002; Agaisse et al., 2003; Hetru et al., 2003). Hemocytes also contribute to both humoral and cellular immune responses by secreting antimicrobial peptides and engulfing and encapsulating foreign invaders (Nappi, 1975; Samakovlis et al., 1990; Franc et al., 1996; Elrod-Erickson et al., 2000). Many mutations have been discovered in *Drosophila* that cause aberrant proliferation and differentiation phenotypes reminiscent of vertebrate blood disorders (Dearolf, 1998). Additionally, these defects are often associated with the production of cellular masses, referred to as melanotic tumors, which blacken due to melanization (Ghelelovitch, 1969; Gateff, 1994).

Drosophila hematopoiesis (Figure 1) first occurs during embryonic development when hemocytes are derived from the head (procephalic) mesoderm and subsequently migrate throughout the embryo (Tepass et al.,

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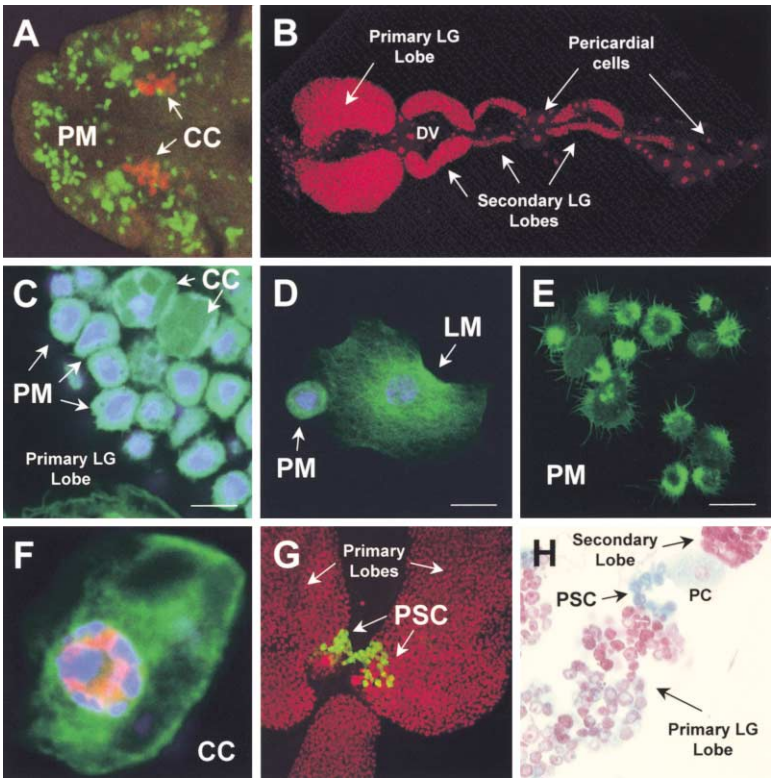

Figure 1. Principal Cell Types and Sites of Hematopoiesis in *Drosophila*

(A) Hemocytes in the developing embryonic head mesoderm (viewed from above, anterior to the left). Merged confocal image of plasmatocytes (green, PM) and crystal cells (red, CC).

(B) The larval lymph gland. Confocal section through a dissected lymph gland (LG, anterior to the left). The bilateral primary and secondary LG lobes, the pericardial cells (nephrocytes), and the dorsal vessel (DV, heart/aorta) bisecting the LG lobes are indicated. Nuclei are marked with TO-PRO-3 (Molecular Probes).

(C) Hemocytes within the primary LG lobe. Confocal section through dissected primary LG lobe in which hemocytes express GFP (green; nuclei are blue). Crystals can be seen in mature crystal cells, which are considerably larger than the plasmatocytes. The scale bar represents 10 μm.

(D) The lamellocyte. Confocal image of a circulating lamellocyte (LM) expressing GFP (green; nuclei are blue). Note the difference in its size with that of a plasmatocyte. The scale bar represents 10 μm.

(E) Plasmatocytes are adhesive and can exhibit filamentous projections. Confocal image of GFP-expressing live larval PMs on a culture slide. Note the flattening of some cells and the cytoplasmic projections.

(F) A mature crystal cell expressing Lz. Confocal image of a circulating crystal cell expressing GFP (green) and an Lz reporter (red). DNA is blue.

(G) The posterior signaling center (PSC) of the lymph gland. Flattened confocal series through the posterior of the primary lymph gland lobes. Cells of the PSC express Serrate (green), which overlaps (yellow) with Srp expression (red) throughout the lymph gland.

(H) Various cell types populate the lymph gland. Plastic section (2 μm) through the LG. PSC cells are indicated in blue. Other cells are differentially counterstained with basic fuchsin. Note different morphologies of blood cells. The densely staining cells in the secondary lobe, and some in the first lobe, are likely to represent prohemocytes. PC, pericardial cell.

1994). A second wave of hematopoiesis is initiated during the larval stage in a specialized organ, termed the lymph gland, which is formed during embryogenesis and persists through the onset of metamorphosis (Rugendorff et al., 1994). During pupariation, the lymph gland degenerates and releases the hemocytes into circulation (Robertson, 1936), in likely anticipation of numerous roles during metamorphosis, including the phagocytic removal of the apoptotic cells generated during the histolysis and remodeling of larval tissues. Although hemocytes are present in the adult fly, no hematopoietic site has been described for this developmental stage.

In *Drosophila*, there are at least three terminally differentiated hemocyte types (Figure 2; Rizki, 1956): plasmatocytes, crystal cells, and lamellocytes. Plasmatocytes are the predominant cell type found at all developmental stages and represent 90%–95% of all hemocytes in circulation, with crystal cells making up the majority of the remainder (Rizki, 1978). Very few lamellocytes are produced under normal conditions; however, large numbers can be induced under specific conditions of immune challenge (Rizki and Rizki, 1992; Lanot et al., 2001; Sorrentino et al., 2002).

In this review, we shall describe the hematopoietic development of *Drosophila* blood cells. Specifically, we attempt to depict the hematopoietic environments as well as the various derived hemocyte types in detail. Furthermore, we discuss the various molecular mechanisms, including transcription factors and signal transduction pathways, known to influence blood development. Last, we compare *Drosophila* hematopoiesis with that of vertebrates in an effort to draw out both similarities and differences.

The Origins of *Drosophila* Hemocytes

Head (Procephalic) Mesoderm

In the embryo, hemocytes differentiate from the head mesoderm and are first identifiable during embryonic stage 5 by their expression of Serpent (Srp), a GATA transcription factor required for hematopoietic development (Tepass et al., 1994; Rehorn et al., 1996; Lebestky et al., 2000). The expression of Srp and their relative position within the embryo define these cells as hemocyte precursors or prohemocytes, which will eventually differentiate into either plasmatocytes or crystal cells (Lebestky et al., 2000). The last cell division within the head mesoderm that gives rise to prohemocytes occurs no later than stage 12, and by the end of embryogenesis all prohemocytes have differentiated (Tepass et al., 1994). Upon maturation, the majority of plasmatocytes migrate out of the head region to populate the embryo in response to specific cues (Tepass et al., 1994). In contrast, crystal cells generally remain localized near their point of origin in the embryo (Figure 1A; Lebestky et al., 2000), although they do subsequently disperse

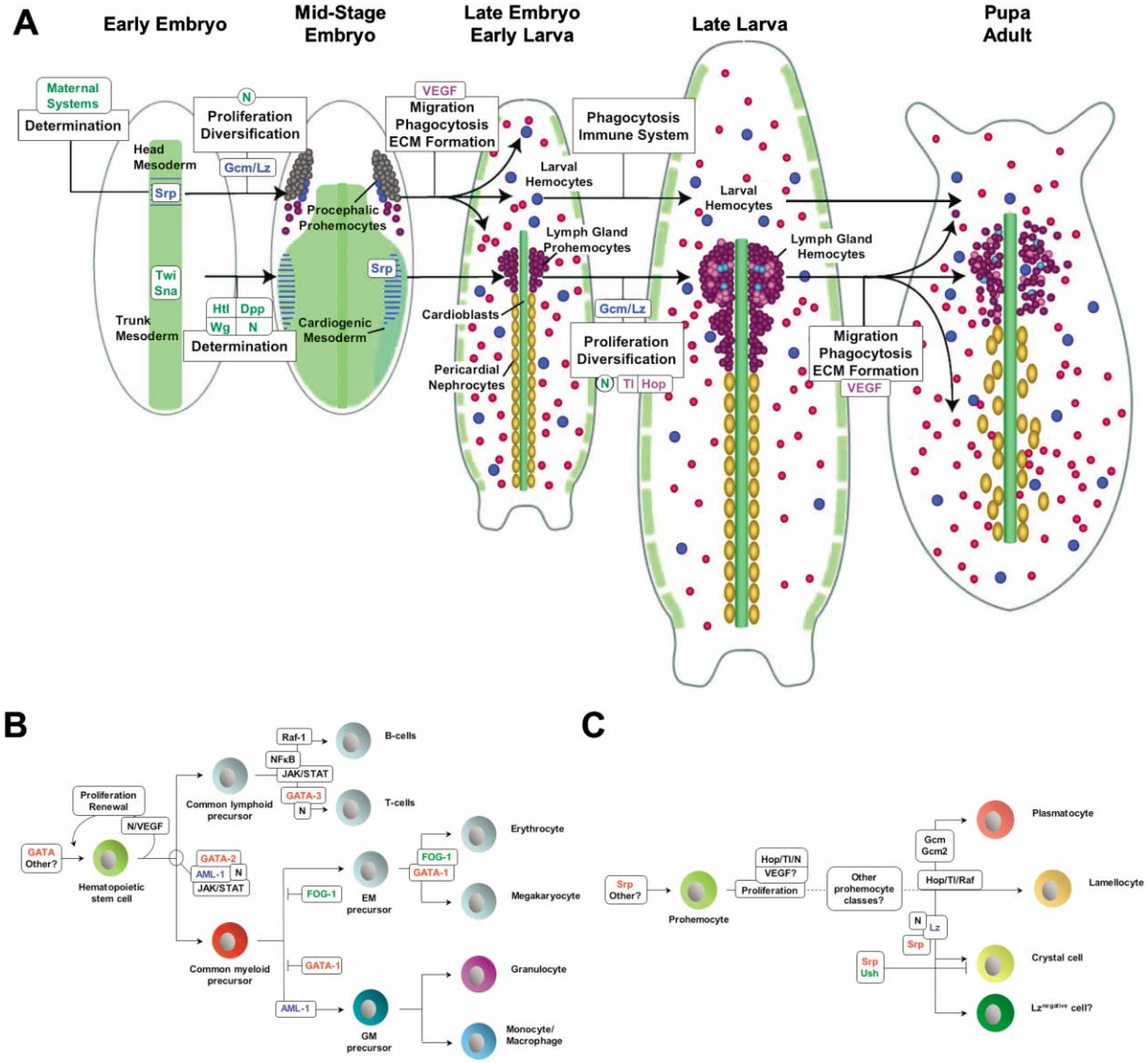

Figure 2. Hematopoietic Development in *Drosophila*

(A) A summary of events during head mesoderm and lymph gland hematopoiesis in *Drosophila*. In the early embryo, expression of the hematopoietic GATA factor Serpent (Srp) within the mesoderm is established by maternal components and the mesoderm-specifying transcription factors Twist (Twi) and Snail (Sna). In midstage embryos, hemocytes are derived from Srp-expressing prohemocytes of the head mesoderm through the function of various factors including Notch (N), the Glial-cells-missing (Gcm) transcription factors 1 and 2, and the Runx factor Lozenge (Lz). During this phase, the cardiogenic mesoderm is specified from the trunk mesoderm by the Heartless (Htl), Decapentaplegic (Dpp), Wingless (Wg), and N signaling pathways. In the late embryo/early larva, mature hemocytes from the head mesoderm have dispersed within the embryo, a process that requires the vascular endothelial growth factor (VEGF) pathway. By this stage, the cardiogenic mesoderm has given rise to the lymph gland, which contains Srp^{+} prohemocytes, the cardioblasts of the dorsal vessel, and the pericardial nephrocytes. By the late larval stage, the lymph gland has expanded through proliferation of Srp^{+} prohemocytes, which likely involves N, Toll (Tl), and Hopscotch (Hop) signaling. Furthermore, lymph gland hemocytes have begun differentiating using N, Gcm, and Lz, as in the head mesoderm. Upon metamorphosis, the lymph gland degenerates through the release of hemocytes into the hemolymph. Subsets of hemocytes derived from both the embryonic head mesoderm and the larval lymph gland will persist through metamorphosis to populate the adult fly.

(B and C) Comparative lineage diagrams of hematopoietic development. A subset of conserved proteins and pathways regulating definitive hematopoiesis in mice (B) are compared with similar cell types, proteins, and pathways in *Drosophila* (C). Lymphoid and ME cells in mice are presented in gray to emphasize that cells of functional similarity are not present in *Drosophila*.

during larval stages. In total, the head mesoderm gives rise to approximately 700 plasmatocytes (Tepass et al., 1994) and 36 crystal cells (A. Milchanowski and U.B., unpublished) by the end of embryogenesis. Interestingly, the number of circulating hemocytes found in the first instar larva was estimated to be significantly less (fewer than 200 per animal); however, by the end of the third larval instar, the total number of hemocytes has significantly expanded to greater than 5,000 cells (Lanot et al., 2001).

Lymph Gland

Besides the head mesoderm, the lateral mesoderm derived from the anterior trunk segments will also eventually give rise to hemocytes (Holz et al., 2003). A small

population of cells called the lymph gland appears in the lateral mesoderm during midembryogenesis and attaches itself to the sides of the dorsal vessel (heart/aorta) after dorsal closure (Rugendorff et al., 1994). As with prohemocytes of the head mesoderm, all lymph gland prohemocytes, including early cells of the lymph gland primordium, express Srp (Lebestky et al., 2000). In contrast to the prohemocytes of the head mesoderm, cells of the lymph gland do not differentiate in the embryo but rather proliferate dramatically during the first half of larval development, forming three to six bilaterally paired cell clusters, or lobes, near the anterior boundary of the abdominal segments (Stark and Marshall, 1930; el Shatoury, 1955). These lymph gland lobes are surrounded by a noncellular layer of ECM and generally decrease in size from anterior to posterior (Figure 1B).

Proliferation of hemocytes in the embryo (both in the head mesoderm and in the lymph gland primordium) follow an intrinsically fixed program where most, if not all, mesodermal cells undergo four cell divisions at specified times (Beer et al., 1987; Bate and Martinez Arias, 1993; Tepass et al., 1994; Klapper et al., 1998; Holz et al., 2003). Hemocytes in the lymph gland subsequently proliferate during the larval growth phase and as such are dependent, as are other larval tissues, on genetic and external factors such as nutrition and, in the specific case of blood cells, immune challenge.

Hemocyte differentiation in the lymph gland can first be observed during the late second larval instar when a small number of prohemocytes in the anterior-most (primary) lymph gland lobes begin to express the crystal cell marker, Lozenge (Lz; Lebestky et al., 2000). By the late third instar, the number of Lz-expressing cells is significantly expanded in the anterior lobes, and a few of these cells can sometimes be found in secondary lobes. In general, hemocyte differentiation remains restricted to the large, primary lobes throughout the majority of the third larval instar, while significant hemocyte differentiation in secondary lobes occurs only near the onset of metamorphosis or during immune challenge.

It is important that the differentiation of hemocytes in the lymph gland should be distinguished from their release into circulation. It has been recurrently suggested that circulating hemocytes present in third instar larvae are derived from the lymph gland, although this has never been rigorously demonstrated, except in the specific case of immune challenge (Lanot et al., 2001; Sorrentino et al., 2002). Contrary to this model, recent investigations employing embryonic transplantation of marked progenitors indicates that hemocytes of the lymph gland are not released into circulation until the onset of metamorphosis, despite ongoing differentiation throughout the third instar (Holz et al., 2003). These studies would suggest that circulating cells in the larval stage are largely, if not entirely, derived from the embryonic head mesoderm that arise through proliferation.

Hematopoiesis in Adult Flies

No hematopoietic tissue has been described in the adult fly, despite the presence of hemocytes in the hemocoel. Whether hematopoietic differentiation occurs in the adult has been difficult to determine; however, transplantation studies using marked blood cell precursors have indicated that at least subsets of the "adult hemocyte" population originate in the embryonic head mesoderm and larval lymph gland (Holz et al., 2003). Phagocytosis assays have demonstrated the presence of plasmatocytes, primarily as sessile cells that tend to accumulate in various locations including the legs, halteres, and near or within the dorsal vessel (Elrod-Erickson et al., 2000; Lanot et al., 2001).

Structural and Functional Characterization of *Drosophila* Hemocytes

Attempts to classify insect hemocytes date back to the nineteenth century and have generally been based on morphological characteristics (Cuenot, 1896; Hollande, 1909; Wigglesworth, 1939; Yeager, 1945; Gupta, 1979). The specific nomenclature for mature *Drosophila* hemocytes was first put forth almost 50 years ago by T.M. Rizki (1956) and includes plasmatocytes, crystal cells, and lamellocytes. The characteristics of each of these *Drosophila* hemocyte types is described in detail below, including molecular markers with which they have subsequently been associated (Figures 1C–1F).

Prohemocytes

Although not a terminal blood cell type, attempts have been made to characterize prohemocytes both morphologically and genetically (Shrestha and Gateff, 1982; Tepass et al., 1994; Rehorn et al., 1996; Lanot et al., 2001). Prohemocytes of the developing head mesoderm as well as the late embryonic and larval lymph gland have been collectively described as cells 4–6 μm in diameter with few defining characteristics. Generally, they have little cytoplasmic volume but do have many free ribosomes and large lipid droplets (Tepass et al., 1994; Lanot et al., 2001). Prohemocytes are also poorly defined with regard to molecular markers. The GATA factor Srp is expressed in prohemocytes of the head mesoderm, but Srp is expressed in parts of the mesoderm that give rise to other cell types, such as fat cells (Rehorn et al., 1996; Sam et al., 1996). Furthermore, Srp expression is not limited to the prohemocyte stage, but is maintained in differentiating and mature hemocytes (Lebestky et al., 2000). Thus, whether true prohemocyte classes exist within the Srp^+ pool awaits the discovery of molecular markers that might distinguish between Srp^+ cells that give rise to blood cells and those that give rise to other tissues.

Plasmatocytes

Plasmatocytes require the expression of the transcription factor Glial-cells-missing (Gcm) for proper differentiation and generally appear as relatively round cells 8–10 μm in diameter with more cytoplasmic volume than is observed in prohemocytes (Shrestha and Gateff, 1982; Bernardoni et al., 1997; Lebestky et al., 2000; Lanot et al., 2001). Additionally, the plasmatocyte cytoplasm usually contains abundant lysosomes and endoplasmic reticulum, which is consistent with their phagocytic and secretory functions. Plasmatocytes function primarily as professional phagocytes and are commonly referred to as macrophages (Rizki, 1978; Shrestha and Gateff, 1982; Tepass et al., 1994; Bangs et al., 2000; Lanot et al., 2001). In this role, plasmatocytes engulf and degrade dead cells and debris as well as invading pathogens. The ability of plasmatocytes to recognize apoptotic cells is mediated by Croquemort (Crq), which is a member of the CD36 family of receptors, which also recognizes apoptotic cells in mammals (Franc et al., 1996). Additionally, a homolog of mammalian phosphatidyl serine receptor has been identified in *Drosophila*, which also

likely functions in the removal of apoptotic cells (Fadok et al., 2000). Pathogen recognition has been shown to be mediated by various receptors, including D-SR-CI and PGRP-LC, which recognize Gram-negative bacteria (Ramet et al., 2001, 2002b). Through the phagocytic removal of cells, plasmatocytes sculpt various developing tissues and organs. An example of this is the embryonic nervous system, which does not condense properly in the absence of plasmatocyte-mediated phagocytosis (Sears et al., 2003). Additionally, flies deficient in plasmatocyte-mediated engulfment exhibit diminished resistance to microbial infection (Elrod-Erickson et al., 2000).

Plasmatocytes also secrete and remodel a wide array of ECM proteins, including Peroxidasin (Pxn), the collagen proteins Dcg1 and Viking (Vkg), as well as Laminin (Fessler et al., 1994; Yasothornsrikul et al., 1997). These proteins are deposited in basement membranes that are critical to many morphogenetic processes (Murray et al., 1995; Kiger et al., 2001). Plasmatocytes also secrete antimicrobial peptides, including Cecropin A1, Drosomycin, and Diptericin, reinforcing the role of the fat body in humoral immunity (Samakovlis et al., 1990; Dimarcq et al., 1997; Roos et al., 1998). Adult hemocytes have also been shown to signal to the fat body by secreting Unpaired-3, a ligand that activates the JAK/STAT pathway in a cytokine-like fashion and promotes antimicrobial peptide synthesis (Agaisse et al., 2003).

Several investigations have noted the presence, primarily at the onset of metamorphosis, of plasmatocyte-like cells exhibiting altered adhesive qualities and extensive cytoplasmic projections or pseudopodia. Collectively, these cells have been referred to as filamentous plasmatocytes or, more generally, podocytes (Rizki and Rizki, 1980; Shrestha and Gateff, 1982). Podocytes are phagocytic and are of similar size to plasmatocytes, and thus it has been suggested that podocytes are a specialized derivation of the plasmatocyte lineage (Rizki, 1962). Furthermore, there is an apparent correlation in the increase in podocyte number with a decrease in plasmatocyte number at the onset of metamorphosis, suggestive of their transformation.

Crystal Cells

Crystal cells are somewhat larger in size (10–12 μm) than plasmatocytes and derive their name from the paracrystalline inclusions they contain (Figures 1C and 1F; Rizki and Rizki, 1980; Shrestha and Gateff, 1982; Lanot et al., 2001). Crystal cells are nonphagocytic and function in the process of melanization, which facilitates innate immune and wound-healing responses (De Gregorio et al., 2002; Ramet et al., 2002a). Molecularly, crystal cells have been defined as hemocytes expressing the transcription factor Lozenge and Prophenoloxidase A1 (Lebestky et al., 2000; Duvic et al., 2002; Fossett et al., 2003).

It is believed that the crystal cell inclusions consist of mass quantities of one or more components of the melanization enzymatic cascade, namely prophenoloxidase enzymes, which are similar to the tyrosinase enzymes of vertebrates (Rizki and Rizki, 1985; Soderhall and Cerenius, 1998). The cell-free hemolymph of *Drosophila* contains phenoloxidase activity that is absent in mutant lines that lack functional crystal cells, and thus it has been proposed that crystal cells supply this activity to the hemolymph (Peeples et al., 1969; Rizki et al., 1980). In other arthropod systems, the production of melanin begins through the activation of a serine protease cascade that converts the zymogen prophenoloxidase to active phenoloxidase (reviewed by Soderhall and Cerenius, 1998). Although not completely described, a similar pathway appears to function in *Drosophila*, where both a candidate serine protease and an inhibitory serpin have been identified (Chosa et al., 1997; De Gregorio et al., 2002; Ligoxygakis et al., 2002). Active phenoloxidase catalyzes the oxidation of phenols to quinones that then nonenzymatically polymerize into melanin. Melanin and its biosynthetic byproducts, such as hydrogen peroxide and nitric oxide, are directly toxic to microorganisms.

Lamellocytes

Lamellocytes are large (15–40 μm across), flat, adherent cells (Figure 1D) that appear to primarily function in the encapsulation and neutralization of objects too large to be engulfed by plasmatocytes (Nappi, 1975; Rizki and Rizki, 1992). These cells are themselves nonphagocytic and their cytoplasmic constituents are relatively sparse (Shrestha and Gateff, 1982; Lanot et al., 2001). Lamellocytes can be identified by their large size and by the expression of an antigen that is recognized by the L1 monoclonal antibody (Asha et al., 2003). Additionally, a *misshapen-lacZ* reporter gene serves as a lamellocyte marker (Braun et al., 1997; Lanot et al., 2001; Sorrentino et al., 2002), although any direct function of the *misshapen* locus, which encodes a component of the JUN kinase signaling cascade, in lamellocyte differentiation is currently unknown.

Very few lamellocytes are observed in normal larvae (Luo et al., 2002); however, significant lamellocyte differentiation is rapidly induced as an immune response. For example, parasitization by the Hymenopteran wasp *Leptopilina boulardi* initiates the rapid differentiation of lamellocytes, which subsequently adhere to and surround (laminate) the egg capsule (Nappi, 1975; Rizki and Rizki, 1992; Carton and Nappi, 2001). Subsequently, this capsule melanizes, locally generating cytotoxic compounds and creating a barrier (Nappi and Vass, 1998; Nappi et al., 2000). Bacterial challenge does not induce lamellocyte differentiation, but the encapsulation response can be invoked artificially with objects placed in the larval hemocoel that are too large to be engulfed (Ratcliffe and Rowley, 1979). Thus, lamellocyte differentiation in the immune context is probably linked to object size and/or the penetration of the larval cuticle and is not pathogen specific. Recently, the gene *Hemese* has been described that, in part, controls lamellocyte differentiation and the encapsulation response (Kurucz et al., 2003). Depletion of Hemese enhances the differentiation of lamellocytes and the encapsulation response upon parasitization, indicating that Hemese normally limits this process. Additionally, depletion of Hemese enhances melanotic tumor formation in the *malignant blood neoplasm-1* tumorous mutant. Although it is generally unclear what seeds their formation, melanotic tumors involve lamellocytes and may represent inappropriate encapsulation responses against self tissues (Rizki and Rizki, 1974, 1979).

Lamellocytes share similar adhesive properties with plasmatocytes, and in vitro studies demonstrate that relatively small, round cells, presumably plasmatocytes,

can be transformed into larger, flattened lamellocyte-like cells, suggesting that lamellocytes represent a further differentiated form of circulating plasmatocytes (Rizki, 1962). In contrast, recent analysis using a molecular marker (*misshapen-lacZ*) suggests that lamellocyte differentiation occurs de novo from precursors within the lymph gland (Lanot et al., 2001; Sorrentino et al., 2002). How signals directing lamellocyte differentiation are communicated from the site of infestation to the lymph gland is not known, although it likely involves initial recognition events by plasmatocytes and/or crystal cells (Russo et al., 1996).

Recently, a gene called *yantar* has been discovered that encodes a novel but conserved protein with a putative RNA-processing function. The mutation of *yantar* exhibits a wide array of hematopoietic effects including aberrant differentiation of lamellocyte-like cells. In *yantar* mutants, the number of cells staining with the lamellocyte-specific antibody L1 is dramatically increased (S. Sinenko and B. Mathey-Prevot, personal communication). However, these cells lack typical lamellocyte morphology and are found to express Lz, which is normally restricted to crystal cells. The finding of cells that express both markers in *yantar* mutants indicates that lamellocytes and crystal cells may share a common progenitor (S. Sinenko and B. Mathey-Prevot, personal communication). It will be interesting to determine whether this result is an explanation for the empirical observation that noncrystal cells can be derived from Lz^+ cells (Lebestky et al., 2000; Figure 2C).

Transcriptional Regulation of Hematopoiesis

Serpent and U-Shaped

Blood cell formation in *Drosophila* commences with the expression of the GATA transcription factor Serpent (Srp), which plays a central role in committing mesodermal cells to hemocyte fate. Serpent (also known as dGATA-B and ABF) is one of five *Drosophila* GATA factor homologs (*pannier, grain*, *dGATA-D*, and *dGATA-E* are the others), but is the only one known to be directly required for hematopoiesis (Rehorn et al., 1996; Lebestky et al., 2000; Patient and McGhee, 2002). The GATA family of zinc finger transcription factors is highly conserved, present from yeast to vertebrates, and has diverse roles in many developmental programs, including cell fate specification, differentiation, and proliferation (Orkin et al., 1998; Lowry and Atchley, 2000; Cantor and Orkin, 2002; Maduro and Rothman, 2002; Patient and McGhee, 2002). In mice, GATA-1, GATA-2, and GATA-3 all have fundamental roles in various aspects of hematopoietic development (Table 1; Pevny et al., 1991; Tsai et al., 1994; Ting et al., 1996; Shivdasani et al., 1997). Furthermore, it has recently been shown that GATA factor function is one of the earliest requirements for the specification of blood progenitors during primitive hematopoiesis (Fujiwara et al., 2003).

In the developing *Drosophila* embryo, the zygotic expression of the transcription factor Twist establishes and is expressed throughout the mesoderm (Castanon and Baylies, 2002). Accordingly, the expression of Srp is downstream of Twist function; however, the timing of Srp expression in the mesoderm is not uniform. Serpent is expressed early in the head mesoderm and the events causing blood cell differentiation closely follow Srp expression in this tissue. In contrast, Srp expression in lymph gland precursors occurs later due to the fact that the generation of these cells is downstream of several signaling inputs and morphogenetic events. Furthermore, there is a relatively long delay between the onset of Srp expression in the lymph gland precursors and the differentiation of these Srp^+ cells into mature hemocytes. During this delay (the entire first half of larval development), cells in the lymph gland undergo extensive proliferation. Once initiated, however, blood cell differentiation in the lymph gland appears to utilize similar genetic mechanisms to those that mediate differentiation of hemocytes in the head mesoderm of the embryo.

It has recently been demonstrated that *srp* primary transcripts are alternatively spliced to generate two protein isoforms, one containing two zinc fingers (SrpNC; N- and C-terminal zinc fingers), similar to vertebrate GATA factors, and the other containing a single zinc finger (SrpC; Waltzer et al., 2002). Functionally, SrpNC was found to exhibit differential DNA binding characteristics compared to SrpC and to differentially regulate the expression of various target genes. Furthermore, SrpNC specifically interacts with the Friend-of-GATA (FOG) homolog U-shaped (Ush) in a manner similar to vertebrate GATA:FOG interactions (Tevosian et al., 1999; Waltzer et al., 2002; Fossett et al., 2003). Interestingly, expression of either Srp isoform in *srp* null embryos is sufficient to support the differentiation of cells expressing plasmatocyte and crystal cell lineage markers, indicating that the N-terminal zinc finger of Srp (and any presumptive interaction with Ush) is not required for the initial steps of hematopoietic specification. However, as described below, Ush is required for subsequent steps of hematopoietic lineage commitment.

U-shaped is a member of the FOG family of zinc finger proteins that modulates the function of GATA transcription factors (Chang et al., 2002). In mice, FOG-1 has been shown to specifically regulate erythropoiesis and megakaryopoiesis in conjunction with GATA-1 (Tsang et al., 1997, 1998; Cantor et al., 2002; Katz et al., 2002), and FOG-2 is required for heart morphogenesis along with GATA-4 (Svensson et al., 1999; Tevosian et al., 1999; Crispino et al., 2001). Likewise, Ush has been shown to interact genetically and physically with Pannier (a homolog of GATA-4) in limiting the production of cardial cells and sensory bristles (Haenlin et al., 1997; Fossett et al., 2000). U-shaped appears to function in a similar manner with Srp during hematopoiesis to repress crystal cell fate, because loss-of-function mutations in *ush* lead to an expansion of the crystal cell population and directed misexpression of Ush in crystal cell precursors causes a reduction in the number of crystal cells (Fossett et al., 2001). U-shaped is expressed in the head mesoderm by embryonic stage 8 and is subsequently maintained in plasmatocytes but is downregulated in crystal cells (Fossett et al., 2001), suggesting that repression of Ush is important for commitment to crystal cell fate. Lineage repression by Ush is likely achieved through interaction with the transcriptional corepressor CtBP, which binds the PXDL motif found in all known FOG proteins (Turner and Crossley, 1998; Holmes et al., 1999; Deconinck et al., 2000; Fossett et al., 2001).

Table 1. Transcriptional Regulators Influencing Hematopoiesis in *Drosophila*

Factor	Function	Vertebrate Homologs	Function	References
Serpent	Hemocyte specification; CC differentiation	Murine GATA-1, -2, -3	Required for primitive hematopoiesis; definitive erythropoiesis, megakaryopoiesis, T cell lymphopoiesis	Pevny et al., 1991; Tsai et al., 1994; Rehorn et al., 1996; Ting et al., 1996; Shivdasani et al., 1997; Lebestky et al., 2000; Fujiwara et al., 2003
U-shaped	Represses CC fate	Murine FOG-1	Required for erythropoiesis and megakaryopoiesis	Tsang et al., 1998; Deconinck et al., 2000; Fosset et al., 2001, 2003
		Xenopus FOG	Represses erythropoiesis	
Lozenge	Specification of CC fate	Murine AML-1/Runx1	Required for definitive hematopoiesis	Okuda et al., 1996; Wang et al., 1996a; Lebestky et al., 2000; Fosset et al., 2003
Gcm/Gcm2	Specification of PM fate	Murine Gcm1, Gcm2	No known hematopoietic function	Bernardoni et al., 1997; Lebestky et al., 2000; Alfonso and Jones, 2002

CC, crystal cell; PM, plasmatocyte.

Lozenge

The transcription factor Lozenge, a known regulator of cell fate specification in the eye (Daga et al., 1996; Flores et al., 1998; Siddall et al., 2003), is also required for crystal cell development (Rizki and Rizki, 1981; Lebestky et al., 2000). Lozenge is a member of the Runx family of transcription factors and has high homology (71% within the Runt domain) to human AML-1/Runx1 (Daga et al., 1996), which is one of the most frequent targets of chromosomal translocations leading to acute myeloid leukemia (AML; Lutterbach and Hiebert, 2000; Speck and Gilliland, 2002). AML-1 forms a heterodimeric complex with CBFβ, a protein homologous to the *Drosophila* proteins Brother and Big brother (Li and Gergen, 1999; Adya et al., 2000; Kaminker et al., 2001; Speck and Gilliland, 2002). Importantly, targeted disruption of either AML-1 or CBFβ leads to a loss of definitive hematopoiesis in mice (Okuda et al., 1996; Sasaki et al., 1996; Wang et al., 1996a, 1996b).

Initially, mutations in *lz* were shown to suppress hemolymph phenoloxidase as well as the *Black cells* (*Bc*) phenotype, in which crystal cells lose their inclusions and become melanized (Peeples et al., 1969; Rizki and Rizki, 1981). Subsequently, it was determined that Lz is required for the hematopoietic differentiation of crystal cells (Lebestky et al., 2000). In the embryo, *lz* expression can first be detected in the head mesoderm at approximately stage 10, which coincides with a functional requirement for Lz in crystal cell development between stages 10 and 14 (Lebestky et al., 2000). Lz is expressed in two bilateral clusters (~18 cells each) within a subset of Srp-expressing prohemocytes in the head mesoderm and defines these cells as crystal cell precursors (Lebestky et al., 2000). By stage 17, these crystal cells have matured into a single, loose cluster that generally remains in the head region. Lozenge expression in the larval lymph gland can first be detected during the late second instar, although when present, this expression is limited to the anterior lobe and consists of only a few cells (Lebestky et al., 2000). By the late third larval instar, many more crystal cell precursors can be observed within the anterior lymph gland lobes and a few are found within the secondary lobes. Lozenge is also expressed in mature crystal cells, both in circulation and in the lymph gland (Figures 1C and 1F).

It has recently been shown that murine Runx1 and GATA-1 physically interact and cooperate in megakaryocyte differentiation in vitro (Elagib et al., 2003), suggesting that Lz could directly interact with Srp in a similar manner during crystal cell differentiation. Coexpression of Lz and Srp throughout the mesoderm causes large numbers of crystal cells to develop, while expression of either alone causes only a modest increase in crystal cell differentiation (Fossett et al., 2003). Furthermore, coexpression of Lz and SrpNC was found to repress *ush* expression, supporting the idea that blocking Ush function is an important step in committing to crystal cell fate. Consistent with this model, pan-mesodermal coexpression of Ush and SrpNC, but not SrpC, specifically blocks crystal cell development. Thus, SrpNC appears to function as a contextual switch, which depends upon the relative levels of *srp*, *lz*, and *ush* expression, in the promotion or repression of the crystal cell lineage (Fossett et al., 2003).

Gcm/Gcm2

Cells entering the plasmatocyte lineage begin to express the transcription factor Glial-cells-missing (Gcm; also known as Glide; Bernardoni et al., 1997; Lebestky et al., 2000) and its homolog Gcm2 (Alfonso and Jones, 2002). The Gcm proteins also function in the nervous system, where they are the primary determinants of glial cell fate (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996; Kammerer and Giangrande, 2001; Alfonso and Jones, 2002). Gcm proteins are novel transcription factors that have been conserved in vertebrates (reviewed by Wegner and Riethmacher, 2001). Two Gcm homologs are found in mammals, though genetic studies have yet to find a role for these genes in vertebrate hematopoiesis (Altshuller et al., 1996; Kim et al., 1998; Gunther et al., 2000; Schreiber et al., 2000). During *Drosophila* blood development, Gcm/Gcm2 expression is restricted to and first detectable in plasmatocyte precursors soon after Srp expression in stage 5 embryos and colocalizes with the plasmatocyte markers Peroxidasin (Pxn) and Croquemort (Crq) during stages 10 and 11 (Bernardoni et al., 1997; Alfonso and Jones, 2002). Gcm/Gcm2 expression diminishes after embryonic stage 11 and is not observed in fully differentiated plasmatocytes.

Embryos mutant for either *gcm* or *gcm2* do not lose

plasmatocytes entirely, but rather exhibit a limited reduction in the number of plasmatocytes (25%–40%; Bernardoni et al., 1997; Alfonso and Jones, 2002). Simultaneous deletion of both *gcm* genes, however, has more severe consequences. All Crq expression is lost and the remaining presumptive plasmatocytes, identified by Pxn expression, are further reduced in number and exhibit aberrant morphologies and migratory behavior. Thus, Gcm and Gcm2 have critical overlapping roles in the early expansion and definitive maturation of the plasmatocyte lineage (Alfonso and Jones, 2002). Gcm/Gcm2 expression is never observed in the crystal cell lineage and their deletion does not affect crystal cell development (Alfonso and Jones, 2002). Gcm is capable, however, of specifying plasmatocyte cell fate when ectopically expressed in crystal cell precursors (Lebestky et al., 2000). Thus, Gcm and Gcm2 appear to function as genetic switches in the specification of plasmatocyte fate, similar to their role in gliogenesis.

Direct targets of Gcm during blood development have not been well established, though several genes have recently been identified that specifically require Gcm/Gcm2 for expression in hemocytes (Freeman et al., 2003). One of these genes, *draper*, is expressed by all Gcm^+ glia and hemocytes and was shown to be required for the phagocytosis of apoptotic cells (Freeman et al., 2003). Interestingly, Draper shares homology with the *C. elegans* CED-1 surface receptor, which is itself required for the engulfment of apoptotic cells in the worm (Zhou et al., 2001). This suggests that the engulfment-mediated cell corpse removal by plasmatocytes as well as that by glia are both controlled by the expression of Gcm/Gcm2.

Chromatin Remodeling Factors

Chromatin modification is a global and relatively generalized mechanism of gene regulation, although many of its effectors have specific roles in hematopoietic development (reviewed by Fisher, 2002; Georgopoulos, 2002). It is now well established that chromatin modification in the form of histone acetylation and deacetylation is associated with transcriptional activation and repression, respectively (see Struhl, 1998; Kurdistani and Grunstein, 2003). The coactivator CREB binding protein (CBP) exhibits histone acetyltransferase (HAT) activity and has many roles in murine hematopoietic development, including interaction with GATA-1 to establish acetylation patterns that promote erythropoiesis (Bannister and Kouzarides, 1996; Letting et al., 2003). CBP has also been shown to critically associate with AML-1 during myeloid differentiation (Kitabayashi et al., 1998). Similarly, *Drosophila* CBP (dCBP) functions during blood formation (Bantignies et al., 2002), though whether dCBP interacts with Srp or Lz remains to be established. Null mutation of dCBP is lethal, though loss of one allele of dCBP has been shown to enhance melanotic tumor formation associated with mutations in *modulo*, which encodes a chromatin-associated factor first identified as a modifier of position effect variegation (Garzino et al., 1992; Bantignies et al., 2002). Interestingly, haploinsufficiency of CBP in humans leads to Rubinstein-Taybi syndrome, which is characterized by various developmental defects and mental retardation and is often accompanied by hematopoietic malignancies including AML (Miller and Rubinstein, 1995; Petrij et al., 1995). Additionally, a significant fraction ($\sim$40%) of mice with targeted disruption of a single allele of CBP were shown to develop an array of hematopoietic defects including tumors and/or tumorigenic cells, which were often associated with a loss of CBP heterozygosity (Kung et al., 2000).

Transcriptional repression via histone deacetylation may also play a role during lineage commitment. Repression of the crystal cell lineage by Ush is likely mediated via interaction with the corepressor CtBP, which is associated with histone deacetylase (HDAC) activity (Sundqvist et al., 1998; Fossett et al., 2001). Similarly, Lz interacts with the corepressor Groucho (TLE1 in mammals; Canon and Banerjee, 2003), which also recruits HDAC activity (Courey and Jia, 2001). Likewise, TLE1 has been shown to interact with AML-1 and to repress AML-1-mediated transactivation (Levanon et al., 1998).

Mutations affecting the function of the nucleosome remodeling complexes SWI/SNF and NURF have also been found to perturb *Drosophila* blood development. The genes *brahma* and *domino* encode members of the SWI2/SNF2 family of DNA-dependent ATPases (the catalytic subunit of the SWI/SNF complex), and both genes are generally required for cell viability and proliferation control (Elfring et al., 1998; Ruhf et al., 2001). As such, mutations in these genes have wide-ranging effects including hematopoietic defects and lethality. Brahma is required to activate hemocyte proliferation while Domino appears to repress the proliferation of hemocytes (Ruhf et al., 2001; Remillieux-Leschelle et al., 2002); neither gene appears crucial for hemocyte differentiation. Similarly, mutation of *iswi*, which encodes a distinct but related DNA-dependent ATPase of the NURF, ACF, and CHRAC chromatin remodeling complexes, causes increased hemocyte proliferation and melanotic tumor formation, and similar results are obtained with mutations in the NURF subunit *nurf301* (Badenhorst et al., 2002). In mammals, SWI/SNF function has been implicated in various hematopoietic processes including the transcription of myeloid- and erythroid-specific genes (Armstrong et al., 1998; Kowenz-Leutz and Leutz, 1999; Lee et al., 1999; Ogawa et al., 2003), and these proteins are often recruited by CBP (Agalioti et al., 2000; Zhang et al., 2001).

Members of the Polycomb (PcG) and trithorax (trxG) groups modify chromatin through the relatively long-term maintenance of previously established transcriptional states (see Lessard and Sauvageau, 2003). Recently, mutation of the PcG gene *multi sex combs* (*mxc*) has been shown to cause hyperproliferation of both circulating and lymph gland hemocytes (Remillieux-Leschelle et al., 2002). Additionally, in *mxc* mutants, the percentage of cells representing podocytes and lamellocytes were found to be increased while those of crystal cells were decreased, compared to wild-type. Several mammalian PcG and trxG genes have been implicated in hematopoiesis including the well-characterized *Mixed-Lineage Leukemia* (*MLL*) gene. *MLL* is a homolog of *trithorax* in *Drosophila* and, like *AML-1*, is a frequent target of chromosomal translocations leading to leukemias in humans and mice (Ayton and Cleary, 2001). Additionally, targeted disruption of *MLL* in mice leads to defects in both primitive and definitive hematopoiesis (Hess et al., 1997; Yagi et al., 1998).

Table 2. Signaling Pathway Components Influencing Hematopoiesis in *Drosophila*

Pathway	Component	Function	Phenotype	References
Notch	Serrate	Ligand	LOF blocks CC differentiation; GOF causes extra CCs	Duvic et al., 2002; Lebestky et al., 2003
	Notch	Receptor	LOF blocks CC differentiation; GOF causes extra CCs	
	Su(H)	Transcriptional regulator	LOF blocks CC differentiation	
JAK/STAT	Hopscotch	JAK kinase	LOF has no clear effect; GOF causes hyperproliferation of hemocytes, excess LM differentiation, melonotic tumor formation	Betz et al., 2001; Hanratty and Dearolf, 1993; Harrison et al., 1995; Luo et al., 1995, 2002; Remillieux-Leschelle et al., 2002
	STAT92E	Transcriptional regulator	LOF inhibits proliferation and LM differentition	
	PIAS	STAT inhibitor	LOF enhances $hop^{Tum\text{-}l}$-mediated tumor formation	
	D-Raf[a]		LOF causes loss of circulating hemocytes and loss of LM differentiation	
Toll/Cactus	Toll	Receptor	LOF causes reduced hemocyte proliferation; GOF causes hyperproliferation of hemocytes, excess LM differentiation, melonotic tumor formation	Gerttula, et al., 1988; Qiu et al., 1998; Remillieux-Leschelle et al., 2002
	Tube	Signaling adapter	LOF causes reduced hemocyte proliferation	
	Pelle	IRAK kinase	LOF causes reduced hemocyte proliferation	
	Cactus	IκB homolog	LOF causes hyperproliferation of hemocytes, excess LM differentiation, melanotic tumor formation	
	Dorsal	NFκB homolog	LOF has no effect; GOF causes melanotic tumor formation	
VEGFR	VEGF-2	Ligand	LOF no clear effect, redundant with VEGF-1/3; GOF can redirect PM migration and can cause excess hemocyte proliferation	Cho et al., 2002; Heino et al., 2001; Munier et al., 2002; Sears et al., 2003
	VEGFR	Receptor	LOF blocks PM migration from head mesoderm	

CC, crystal cell; LM, lamellocyte; PM, plasmatocyte; LOF, loss of function; GOF, gain of function; Su(H), Suppressor of Hairless.
[a] See text.

Signal Transduction Pathways Influencing Hematopoiesis

The Notch Pathway

In *Drosophila*, the Notch (N) pathway has been shown to have an important role in lineage specification (Table 2). Loss of N signaling blocks the expression of Lz (Lebestky et al., 2003) and prophenoloxidase (Duvic et al., 2002), while ectopic expression of an activated form of N dramatically increases the number of Lz^+ cells within the lymph gland, particularly in secondary lobes where few Lz-expressing cells are normally found (Lebestky et al., 2003). Notch signaling is similarly required for crystal cell development in the head mesoderm (Lebestky et al., 2003).

Of the two known ligands for the N receptor in *Drosophila*, Serrate (Ser) and Delta, only Ser appears to function during hematopoiesis. Examination of Ser expression in the lymph gland identified a cluster of Ser^+ cells near the posterior tip of the primary (anterior-most) lobe, designated the posterior signaling center (PSC; Figure 1G; Lebestky et al., 2003). Other Ser^+ cells, which may be derived from the PSC, are often scattered within the primary lobes, where most crystal cells develop. Cells of the PSC do not express Lz and do not require N signaling for their own development. Additionally, PSC cells rarely divide in comparison with other cells of the lymph gland. Thus, the hematopoietic microenvironment of the lymph gland appears functionally similar to that of vertebrates. Both contain a signaling cell population, the PSC in the lymph gland, and, for example, the stromal cells of the mammalian bone marrow, as well as blood cells that differentiate in response to those signals. In this context, it is interesting to note that murine bone marrow stromal cells have been found to express the mammalian Ser homolog Jagged-1 (Lindsell et al., 1995), which influences the proliferation and differentiation of various hematopoietic precursors (Varnum-Finney et al., 1998).

In mice and humans, N has been implicated in the regulation of various hematopoietic processes (reviewed by Allman et al., 2002; Radtke et al., 2002). Expression analyses have shown that N is selectively utilized during various stages of hematopoiesis (Walker et al., 2001). Activation of N in bone marrow hematopoietic stems cell (HSCs) in vitro leads to increased proliferation and survival (Varnum-Finney et al., 1998; Karanu et al., 2000). Additionally, signaling via Notch1 is required for the generation of HSCs from endothelial cells in mice (Kumano et al., 2003) and also controls the differentiation of myeloid cells from HSCs by regulating GATA-2

expression (Kumano et al., 2001). Notch signaling is also reiteratively utilized during lymphocyte development, where it mediates commitment to the T cell lineage (Pui et al., 1999; Radtke et al., 1999; Wilson et al., 2001). Subsequently, N mediates the determination of T cell type, $\alpha\beta$ versus $\gamma\delta$ and CD4 versus CD8 (Robey et al., 1996; Washburn et al., 1997; Fehling et al., 1999). It has also been shown that the B cell lineage specifically utilizes Notch2 signaling during marginal zone B cell development (Saito et al., 2003). Last, excessive activation the N signaling pathway has been associated with increased leukemogenic potential in the T cell lineage (reviewed by Aster and Pear, 2001).

The VEGF Receptor Pathway

The vascular endothelial growth factor receptor (VEGFR, also known as PDGF/VEGF receptor or PVR in *Drosophila*) signaling pathway has previously been implicated in the control of hemocyte migration (Heino et al., 2001; Cho et al., 2002; Sears et al., 2003), similar to its role in border cell migration during oogenesis (Duchek et al., 2001). The single *Drosophila* VEGF receptor is first expressed in prohemocytes of the head mesoderm at stage 8 and is subsequently maintained in plasmatocytes (Heino et al., 2001; Cho et al., 2002). Despite the early expression of VEGFR/PVR, its function is not required for plasmatocyte specification or differentiation (Heino et al., 2001; Cho et al., 2002; Sears et al., 2003). Rather, VEGFR mutant plasmatocytes, once mature, do not properly migrate within the embryo. Consistent with this proposed role in hemocyte migration, each of the three *Drosophila* VEGF ligands is expressed, subsequent to VEGFR/PVR expression, in tissues along these routes where they have been hypothesized to act as migration cues (Cho et al., 2002).

Recent analysis of VEGFR/PVR signaling, however, suggests that the observed plasmatocyte migration defect could primarily be a consequence of diminished hemocyte survival. Disruption of the VEGFR/PVR leads to a slow but continuous reduction in the number of plasmatocytes through apoptotic cell death during embryonic development (K. Brückner and N. Perrimon, personal communication). Similarly, abolishing VEGFR/PVR expression in a hemocyte-like cell line leads to cell death in vitro, which is consistent with similar experiments where blocking VEGFR signaling was found to stop cell proliferation (Munier et al., 2002). Much of the hemocyte cell death and migration defects can be suppressed by the activation of downstream effectors of VEGFR/PVR signaling as well as ectopic expression of an inhibitor of apoptosis (K. Brückner and N. Perrimon, personal communication). Thus, it appears that VEGFR/PVR signaling provides an essential trophic survival cue for hemocytes that ultimately has an effect on their migration throughout the embryo (Heino et al., 2001; Cho et al., 2002; Sears et al., 2003).

In mammals, the VEGFR pathway has primarily been associated with regulation of vascular endothelial cells during the processes of angiogenesis and vasculogenesis, both in normal development and in pathological scenarios such as tumor growth (reviewed by Ferrara et al., 2003). With regard to blood development, signaling through the VEGFR pathway exhibits various effects including the promotion of B cell development and inhibition of dendritic cell maturation (Gabrilovich et al., 1996; Hattori et al., 2001). Interestingly, conditional disruption of VEGF in bone marrow HSCs blocks their ability to repopulate lethally irradiated mice, though signaling through VEGFR restores this ability, implicating a VEGF autocrine signaling loop in the maintenance and/or survival of HSCs (Gerber et al., 2002). Last, increased expression of VEGF ligands as well as receptors have been associated with a large number of hematological malignancies, suggestive of their role in promoting development and/or proliferation (Ferrara et al., 2003).

The JAK/STAT Pathway

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signal transduction pathway is also conserved in *Drosophila* and it modulates many developmental processes including hemocyte proliferation and differentiation (reviewed by Hou et al., 2002). Mutations that cause constitutive activation of the JAK kinase Hopscotch (such as $hop^{Tum\text{-}l}$ and hop^{T42}) lead to a leukemia-like hyperproliferation of both circulating and lymph gland hemocytes during larval stages and facilitate the formation of melanotic tumors (Harrison et al., 1995; Luo et al., 1995). Transplantation studies have demonstrated the cell-autonomous nature of these mutations through the clonal expansion of mutant hemocytes and formation of tumors within wild-type hosts (Hanratty and Ryerse, 1981; Luo et al., 1995). Hyperactivation of Hop primarily affects plasmatocytes, which are expanded in number and exhibit altered morphologies, though lamellocyte differentiation is also increased by the $hop^{Tum\text{-}l}$ and hop^{T42} mutations, facilitating tumor formation. Interestingly, crystal cells appear unaffected in these mutant backgrounds (Silvers and Hanratty, 1984), suggesting that *hop* or downstream effectors are downregulated in this lineage. In fact, misexpression of the activated Hop protein in crystal cell precursors will suppress crystal cell fate (Lebestky, 2001). Null mutations of *hop* result in an underproliferation of diploid tissues and often give rise to late larval/pupal lethal phenotypes (Perrimon and Mahowald, 1986), though examination of circulating as well as lymph gland hemocytes revealed no significant defect due to zygotic *hop* deficiency (Remillieux-Leschelle et al., 2002). Thus, JAK signaling is either not required for normal hematopoietic development or maternal Hop has sufficient activity to support blood development.

Drosophila STAT (D-STAT/STAT92E/Marelle) is hyperactivated in a $hop^{Tum\text{-}l}$ background and is required for $hop^{Tum\text{-}l}$-mediated plasmatocyte proliferation, lamellocyte differentiation, and tumor formation (Luo et al., 2002; Remillieux-Leschelle et al., 2002). Accordingly, deficiency in *Drosophila* PIAS, an inhibitor of STAT function, enhances melanotic tumor formation (Betz et al., 2001). Interestingly, one target of activated D-STAT is the promoter of *Drosophila* Raf (D-Raf), a member of the canonical Ras/Raf/MAP kinase pathway (Kwon et al., 2000). D-Raf has been shown to physically interact with Hop and is required for both normal and $hop^{Tum\text{-}l}$-mediated lamellocyte differentiation as well as the proliferation or survival of circulating hemocytes (Luo et al., 2002). Expression of activated Ras in hemocytes causes hyperproliferation as well as the transcriptional modulation of many genes, but this is also suppressed by a loss of D-Raf (Asha et al., 2003). Thus, the Hop and Ras pathways cooperate in the control of hemocyte

proliferation and differentiation. Signaling downstream of Hop also utilizes chromatin remodeling factors to implement transcriptional programs. Mutations in *brahma* suppress $hop^{Tum\text{-}l}$-mediated hyperproliferation while mutations in the NURF remodeling complex enhance tumor formation while specifically derepressing the expression of Hop target genes (Badenhorst et al., 2002; Remillieux-Leschelle et al., 2002).

In vertebrates, the JAK/STAT pathway has been associated with several aspects of hematopoietic development (Rane and Reddy, 2002). Murine JAK2 is required for erythropoiesis and chromosomal translocations within JAK2, and the transcription factor Tel has been associated with leukemia (Lacronique et al., 1997; Peeters et al., 1997; Neubauer et al., 1998; Parganas et al., 1998). Murine STAT5, which is most similar to D-STAT, is required for Tel/JAK leukemic transformation (Schwaller et al., 2000), and the hyperactivation of STAT homologs has been associated with various cancers including leukemias and lymphomas (Bromberg, 2002). Furthermore, STAT5 is required in hematopoietic progenitors and for proper myeloid cell function (Kieslinger et al., 2000). Last, murine Raf-1 facilitates hematopoietic cell survival as well as promotes B cell development (Iritani et al., 1997; Mikula et al., 2001; Haughn et al., 2003).

The Toll/Cactus Pathway

The Toll (Tl) signal transduction pathway was first shown to control dorsal/ventral patterning during embryogenesis (Anderson et al., 1985) and, subsequently, to be important for humoral immunity (Lemaitre et al., 1995; Rosetto et al., 1995; Ip and Levine, 1994). Additionally, mutations that cause constitutive activation of the Tl pathway lead to hematopoietic phenotypes similar to those observed for the JAK/STAT pathway, including the expansion of the plasmatocyte and lamellocyte populations as well as melanotic tumor formation (Gerttula et al., 1988; Qiu et al., 1998). In contrast, loss-of-function mutations in Tl as well as downstream effectors both reduce the number of circulating hemocytes and suppress gain-of-function-mediated melanotic tumor formation (Qiu et al., 1998), further implicating this pathway in proliferation control. Last, Tl signaling is normally antagonized by the PcG protein Mxc, because mutations in *mxc* lead to hematopoietic defects that are suppressed by loss-of-function mutations in the Tl pathway (Remillieux-Leschelle et al., 2002).

Drosophila Toll is a transmembrane receptor that controls the activity of the Rel transcription factors Relish, Dorsal, and Dif, which share homology with mammalian NFκB proteins (Hashimoto et al., 1988; Schneider et al., 1991; Rosetto et al., 1995; Govind, 1999; Silverman and Maniatis, 2001). Cactus (an IκB homolog) functions as an inhibitor of Rel transcription factors by sequestering these proteins in the cytoplasm. Toll signaling causes the degradation of Cactus, thereby allowing its Rel counterpart to enter the nucleus and regulate transcription. Gain-of-function mutations in *Tl* (such as Tl^{10b}) and loss-of-function mutations in *cactus* (such as $cactus^{A2}$) both cause increased nuclear Rel activity and hematopoietic defects (Gerttula et al., 1988; Belvin et al., 1995; Qiu et al., 1998). Although Tl function in *Drosophila* hemocyte proliferation has been well established, the identity of the Rel protein(s) involved is less clear. Dorsal is expressed in hemocytes, but loss of *dorsal* fails to suppress $cactus^{A2}$-mediated hyperproliferation and tumor formation (Qiu et al., 1998), suggesting that this protein is redundant with Relish and/or Dif. As mentioned, hyperactivation of Tl also causes significant lamellocyte differentiation, similar to hyperactivation of the JAK/STAT pathway. Moreover, loss of a single copy of D-STAT suppresses the Tl^{10B}-mediated lamellocyte differentiation (Remillieux-Leschelle et al., 2002), suggesting that there is crosstalk between the Toll and JAK/STAT pathways during lamellocyte differentiation.

In *Drosophila*, the Toll pathway regulates development, immunity, and hematopoiesis (Anderson et al., 1985; Lemaitre et al., 1995; Qiu et al., 1998). In mammals, this pathway primarily mediates innate immune responses through an array of Toll-like receptors (TLRs) that respond to various microbial stimuli (Silverman and Maniatis, 2001). Signaling through numerous receptors, including the TLRs and the tumor necrosis factor receptor, result in the activation of NFκB proteins, which mediate immune and hematopoietic responses. Targeted disruption of various murine NFκB/IκB components causes a variety of hematopoietic disorders (reviewed by Gerondakis et al., 1999), including proliferation and differentiation defects. Not surprisingly, these hematopoietic deficiencies are often associated with defects in innate and adaptive immune responses. Interestingly, targeted disruption of IκBα causes extensive granulopoiesis (Beg et al., 1995), reminiscent of the overproliferation of hemocytes in *cactus* mutants of *Drosophila* (Qiu et al., 1998). Last, many cancers, including a large number of leukemias and lymphomas, have been associated with the dysfunction of NFκB/IκB genes (see Rayet and Gelinas, 1999).

Perspectives on Blood Development in *Drosophila* and Vertebrates

As described above, hematopoiesis in *Drosophila* occurs in two distinct temporal waves: one in the embryo and another during larval development. Additionally, these waves occur in the spatially distinct locations of the embryonic head mesoderm and the larval lymph gland. This reinitiation and relocation of hematopoiesis in *Drosophila* is similar to what is seen during vertebrate primitive and definitive hematopoietic stages (Figure 2A; Traver and Zon, 2002). Overall, *Drosophila* hematopoiesis generates cells with functional similarity to cells of the granulocyte/macrophage branch of the vertebrate lineage (Figures 2B and 2C). Furthermore, head mesoderm hematopoiesis in *Drosophila* is rather similar to primitive hematopoiesis in vertebrates in that both primarily provide a developmental function during embryogenesis. In contrast, immune functions become more important in later stages of development. In both *Drosophila* and mammals, embryonic development is protected from the environment, and thus pathogens are not usually encountered until hatching in the case of *Drosophila*, or birth in vertebrates. Thus, secondary periods of hematopoiesis, in the lymph gland of *Drosophila* larvae and definitive hematopoiesis in vertebrates, generate large populations of cells important for immunity.

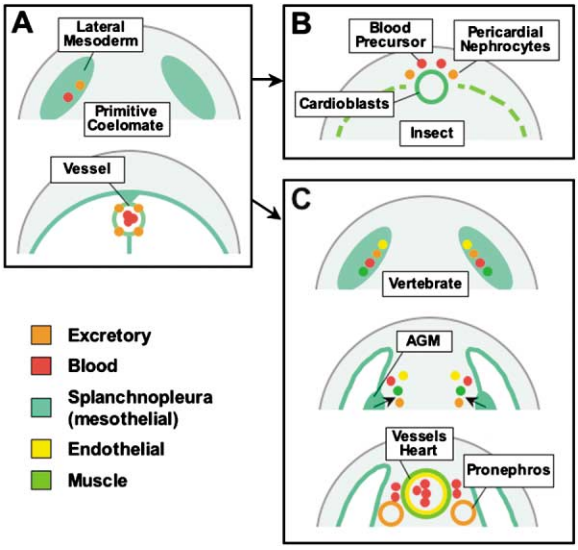

Figure 3. An Evolutionary View of the Development of Hematopoietic, Vascular, and Excretory Mesodermal Components

Primitive coelomates (A), insects (B), and mammals (C). See text for details.

It is interesting to speculate that hematopoiesis in the head mesoderm of *Drosophila* and primitive hematopoiesis in the yolk sac of mammals represent related processes that have undergone a developmental transition during evolution. Functionally and developmentally primitive macrophages in vertebrates are similar to *Drosophila* plasmatocytes. Unlike the macrophages derived from definitive hematopoiesis, primitive macrophages in mice retain proliferative capacity subsequent to differentiation (Takahashi et al., 1989; Takahashi and Naito, 1993), as is seen with plasmatocytes. Moreover, both plasmatocytes and primitive macrophages are thought to be important for sculpting and remodeling of tissues during development. The relationship between head mesoderm hematopoiesis in *Drosophila* and primitive hematopoiesis in vertebrates is further highlighted in lower vertebrates such as zebrafish and *Xenopus*. Like *Drosophila* plasmatocytes, primitive macrophages in zebrafish originate in the mesoderm of the head region, though these cells subsequently migrate to the yolk sac where they mature, as in mice (Herbomel et al., 1999). Once mature, primitive macrophages leave the yolk sac to colonize the anterior region of the embryo proper, where they begin to remove apoptotic cells. Similar primitive macrophages have been shown to be derived from the head region of *Xenopus* (Ohinata et al., 1990).

As a further developmental correlate, a high degree of similarity is apparent between lymph gland development in *Drosophila* and definitive hematopoiesis that occurs in the AGM region of mammals. *Drosophila* lymph gland progenitors develop from a subregion of the lateral mesoderm, termed the cardiogenic mesoderm, which also gives rise to vascular and excretory cells (Figure 3). The specification of the cardiogenic mesoderm requires the input of FGF, Dpp, and Wg signaling, and is inhibited by N signaling (Frasch, 1995; Wu et al., 1995; Gisselbrecht et al., 1996; Bodmer and Venkatesh, 1998; Cripps and Olson, 2002; L. Mandal, U.B., and V.H., unpublished). Progenitor cells of the blood, cardiovascular, and excretory systems are also closely related molecularly and developmentally in the mesodermal AGM domain of vertebrates. In remarkable similarity to the situation in *Drosophila*, differentiation is induced in response to multiple converging signaling pathways, including FGF, BMP, and Wnt (see Bodmer and Venkatesh, 1998; Cripps and Olson, 2002).

The significant similarities in the way in which blood cells develop in arthropods and vertebrates, and the close relationship blood precursors have to cells of the vascular and excretory system in both animal groups, prompt the question of whether these systems might be considered homologous. In other words, did the common Bilaterian ancestor possess blood cells, blood vessels, and excretory organs that developed in close proximity to each other? Comparative morphological evidence, not contradicted by molecular phylogenetic data, suggests that the Bilaterian ancestor was most likely a small acoelomate or pseudocoelomate worm similar to extant "lower" invertebrates, such as platyhelminths (flatworms) or nemathelminths (Gerhart and Kirschner, 1997). A specialized vascular system or respiratory system was probably absent, though cells specialized for transport and excretions were likely present because they exist in most extant Bilaterian phyla. One can further assume that groups of mesoderm cells in the Bilaterian ancestor could have formed epithelial structures lining internal tubules or cavities.

A vascular system made its first appearance in coelomate worms, including both protostome taxa (e.g., annelids) and deuterostome taxa (e.g., hemichordates and cephalochordates). In coelomates, the mesoderm transforms into an epithelial sac, the walls of which attach to the ectoderm (somatopleura) and the inner organs (splanchnopleura). Blood vessels are formed by tubular clefts bounded by the splanchnopleura (Figure 3A). The specialized splanchnopleural cells forming the vascular tubes between them are contractile, myo-epithelial cells and thereby closely resemble cardioblasts in insects, to which they have indeed been homologized in a number of classical studies. Excretory nephrocytes are integrated into the vascular walls, which also gives rise to blood cells circulating within the blood vessels. Thus, blood, vascular, and excretory cells in these animals were closely related and formed a single, functional system.

Further evolutionary changes separated the three systems, but, as we pointed out in this review, the close relationship is still evidenced developmentally and by the existence of shared molecular mechanisms. In arthropods, blood, vascular, and excretory cells derive from an equivalence group, the cardiogenic mesoderm (Figure 3B). The different cell types are separated from each other during embryogenesis, but stay in close contact for much of development (e.g., lymph gland and pericardial nephrocytes lined up next to the dorsal vessel). In vertebrates, the AGM region gives rise to both endothelial and blood precursors that migrate to various sites within the embryo to assemble into blood vessels and diversify into separate hematopoietic lineages, respectively (Figure 3C). The rest of the AGM forms the mesonephros, which in many ways still resembles the excretory nephridia of invertebrate coelomates.

Despite the obvious developmental and morphological differences in the body plan of *Drosophila* and higher vertebrates, many fundamental mechanisms of genetic control are conserved between them. A clear example of this is the organization and expression of the homeotic gene clusters that pattern the anterior-posterior (A/P) axis. At the molecular level, the homeotic genes are organized in generally the same linear order along the chromosome in both *Drosophila* and vertebrates and are correspondingly expressed in overlapping patterns along their respective A/P axes (Kmita and Duboule, 2003). Eye formation offers an additional example of conserved molecular genetic mechanisms. Although the fully developed *Drosophila* eye shows little similarity to the vertebrate eye, the function of a complex of proteins including Pax-6 is crucial for the development of either eye tissue (Gehring, 2002). Often, tissues such as vertebrate and *Drosophila* wings are not homologous, yet they follow a similar logic of development that includes a common network of genes (Martin, 2001). Therefore, the limited cell types and functions of *Drosophila* blood, compared to the relative complexity of mammalian blood, should not be viewed as a deterrent to using *Drosophila* as a model system of hematopoietic development. In fact, the conserved function of GATA/Runx/FOG-like transcription factors, the similar use of signaling pathways, and the apparent similarities in developmental strategies alluded to in this review all suggest that the use of *Drosophila* as a model system to study hematopoietic development offers great potential for the identification and characterization of novel genes and functions that are relevant to vertebrate systems. Furthermore, *Drosophila* should be useful in further characterizing several factors already known to be important for vertebrate hematopoiesis. For example the potential hematopoietic roles of *Drosophila* SCL (HLH3B) and *Drosophila* Lmo (Beadex) await investigation, while the role of *Drosophila* Myb in hemocyte proliferation and differentiation is being determined (Davidson et al., 2003). With the current effort in the development of new tools and genetic markers specific for the *Drosophila* hematopoietic system, new genetic screens can be devised to facilitate the discovery of regulatory genes and mechanisms. It is hoped that the ability to manipulate the function of such genes in *Drosophila* will aid in understanding function and dysfunction in human hematopoiesis.

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References

Adya, N., Castilla, L.H., and Liu, P.P. (2000). Function of CBFβ/Bro proteins. Semin. Cell Dev. Biol. *11*, 361–368.

Agaisse, H., Petersen, U.M., Boutros, M., Mathey-Prevot, B., and Perrimon, N. (2003). Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. Dev. Cell *5*, 441–450.

Agalioti, T., Lomvardas, S., Parekh, B., Yie, J., Maniatis, T., and Thanos, D. (2000). Ordered recruitment of chromatin modifying and general transcription factors to the IFN-β promoter. Cell *103*, 667–678.

Akashi, K., Traver, D., Miyamoto, T., and Weissman, I.L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature *404*, 193–197.

Alfonso, T.B., and Jones, B.W. (2002). gcm2 promotes glial cell differentiation and is required with glial cells missing for macrophage development in *Drosophila*. Dev. Biol. *248*, 369–383.

Allman, D., Aster, J.C., and Pear, W.S. (2002). Notch signaling in hematopoiesis and early lymphocyte development. Immunol. Rev. *187*, 75–86.

Altshuller, Y., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., and Frohman, M.A. (1996). Gcm1, a mammalian homolog of *Drosophila* glial cells missing. FEBS Lett. *393*, 201–204.

Anderson, K.V., Bokla, L., and Nusslein-Volhard, C. (1985). Establishment of dorsal-ventral polarity in the *Drosophila* embryo: the induction of polarity by the Toll gene product. Cell *42*, 791–798.

Armstrong, J.A., Bieker, J.J., and Emerson, B.M. (1998). A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF in vitro. Cell *95*, 93–104.

Asha, H., Nagy, I., Kovacs, G., Stetson, D., Ando, I., and Dearolf, C.R. (2003). Analysis of ras-induced overproliferation in *Drosophila* hemocytes. Genetics *163*, 203–215.

Aster, J.C., and Pear, W.S. (2001). Notch signaling in leukemia. Curr. Opin. Hematol. *8*, 237–244.

Ayton, P.M., and Cleary, M.L. (2001). Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. Oncogene *20*, 5695–5707.

Badenhorst, P., Voas, M., Rebay, I., and Wu, C. (2002). Biological functions of the ISWI chromatin remodeling complex NURF. Genes Dev. *16*, 3186–3198.

Bangs, P., Franc, N., and White, K. (2000). Molecular mechanisms of cell death and phagocytosis in *Drosophila*. Cell Death Differ. *7*, 1027–1034.

Bannister, A.J., and Kouzarides, T. (1996). The CBP co-activator is a histone acetyltransferase. Nature *384*, 641–643.

Bantignies, F., Goodman, R.H., and Smolik, S.M. (2002). The interaction between the coactivator dCBP and Modulo, a chromatin-associated factor, affects segmentation and melanotic tumor formation in *Drosophila*. Proc. Natl. Acad. Sci. USA *99*, 2895–2900.

Bate, M., and Martinez Arias, A. (1993). The Development of *Drosophila melanogaster* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Beer, J., Technau, G.M., and Campos-Ortega, J.A. (1987). Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster*. IV. Commitment and proliferative capabilities of mesodermal cells. Rouxs Arch. Dev. Biol. *196*, 222–230.

Beg, A.A., Sha, W.C., Bronson, R.T., and Baltimore, D. (1995). Constitutive NF-κ B activation, enhanced granulopoiesis, and neonatal lethality in I κ B α-deficient mice. Genes Dev. *9*, 2736–2746.

Belvin, M.P., Jin, Y., and Anderson, K.V. (1995). Cactus protein degradation mediates *Drosophila* dorsal-ventral signaling. Genes Dev. *9*, 783–793.

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

Betz, A., Lampen, N., Martinek, S., Young, M.W., and Darnell, J.E., Jr. (2001). A *Drosophila* PIAS homologue negatively regulates stat92E. Proc. Natl. Acad. Sci. USA *98*, 9563–9568.

Bodmer, R., and Venkatesh, T.V. (1998). Heart development in *Drosophila* and vertebrates: conservation of molecular mechanisms. Dev. Genet. *22*, 181–186.

Braun, A., Lemaitre, B., Lanot, R., Zachary, D., and Meister, M.

(1997). *Drosophila* immunity: analysis of larval hemocytes by P-element-mediated enhancer trap. Genetics *147*, 623–634.

Bromberg, J. (2002). Stat proteins and oncogenesis. J. Clin. Invest. *109*, 1139–1142.

Campos-Ortega, J.A., and Hartenstein, V. (1997). The Embryonic Development of *Drosophila melanogaster*, Second Edition (Berlin, Heidelberg: Springer-Verlag).

Canon, J., and Banerjee, U. (2003). In vivo analysis of a developmental circuit for direct transcriptional activation and repression in the same cell by a Runx protein. Genes Dev. *17*, 838–843.

Cantor, A.B., and Orkin, S.H. (2002). Transcriptional regulation of erythropoiesis: an affair involving multiple partners. Oncogene *21*, 3368–3376.

Cantor, A.B., Katz, S.G., and Orkin, S.H. (2002). Distinct domains of the GATA-1 cofactor FOG-1 differentially influence erythroid versus megakaryocytic maturation. Mol. Cell. Biol. *22*, 4268–4279.

Carton, Y., and Nappi, A.J. (2001). Immunogenetic aspects of the cellular immune response of *Drosophila* against parasitoids. Immunogenetics *52*, 157–164.

Castanon, I., and Baylies, M.K. (2002). A Twist in fate: evolutionary comparison of Twist structure and function. Gene *287*, 11–22.

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.

Chosa, N., Fukumitsu, T., Fujimoto, K., and Ohnishi, E. (1997). Activation of prophenoloxidase A1 by an activating enzyme in *Drosophila melanogaster*. Insect Biochem. Mol. Biol. *27*, 61–68.

Courey, A.J., and Jia, S. (2001). Transcriptional repression: the long and the short of it. Genes Dev. *15*, 2786–2796.

Cripps, R.M., and Olson, E.N. (2002). Control of cardiac development by an evolutionarily conserved transcriptional network. Dev. Biol. *246*, 14–28.

Crispino, J.D., Lodish, M.B., Thurberg, B.L., Litovsky, S.H., Collins, T., Molkentin, J.D., and Orkin, S.H. (2001). Proper coronary vascular development and heart morphogenesis depend on interaction of GATA-4 with FOG cofactors. Genes Dev. *15*, 839–844.

Cuenot, L. (1896). Etudes physiologiques sur les Orthopteres. Arch. Biol. *14*, 293–341.

Daga, A., Karlovich, C.A., Dumstrei, K., and Banerjee, U. (1996). Patterning of cells in the *Drosophila* eye by Lozenge, which shares homologous domains with AML1. Genes Dev. *10*, 1194–1205.

Davidson, C.J., Tirouvanziam, R., and Lipsick, J.S. (2003). Flow cytometry demonstrates a role for Dm-Myb in *Drosophila* blood cell function and proliferation. Annu. Dros. Res. Conf. *44*, 932B.

Dearolf, C.R. (1998). Fruit fly "leukemia." Biochim. Biophys. Acta *1377*, M13–M23.

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.

De Gregorio, E., Han, S.J., Lee, W.J., Baek, M.J., Osaki, T., Kawabata, S., Lee, B.L., Iwanaga, S., Lemaitre, B., and Brey, P.T. (2002). An immune-responsive Serpin regulates the melanization cascade in *Drosophila*. Dev. Cell *3*, 581–592.

Dimarcq, J.L., Imler, J.L., Lanot, R., Ezekowitz, R.A., Hoffmann, J.A., Janeway, C.A., and Lagueux, M. (1997). Treatment of l(2)mbn *Drosophila* tumorous blood cells with the steroid hormone ecdysone amplifies the inducibility of antimicrobial peptide gene expression. Insect Biochem. Mol. Biol. *27*, 877–886.

Duchek, P., Somogyi, K., Jekely, G., Beccari, S., and Rorth, P. (2001). Guidance of cell migration by the *Drosophila* PDGF/VEGF receptor. Cell *107*, 17–26.

Duvic, B., Hoffmann, J.A., Meister, M., and Royet, J. (2002). Notch signaling controls lineage specification during *Drosophila* larval hematopoiesis. Curr. Biol. *12*, 1923–1927.

Dzierzak, E. (1999). Embryonic beginnings of definitive hematopoietic stem cells. Ann. NY Acad. Sci. *872*, 256–262; discussion 262–254.

Elagib, K.E., Racke, F.K., Mogass, M., Khetawat, R., Delehanty, L.L., and Goldfarb, A.N. (2003). RUNX1 and GATA-1 coexpression and cooperation in megakaryocytic differentiation. Blood *101*, 4333–4341.

Elfring, L.K., Daniel, C., Papoulas, O., Deuring, R., Sarte, M., Moseley, S., Beek, S.J., Waldrip, W.R., Daubresse, G., DePace, A., et al. (1998). Genetic analysis of brahma: the *Drosophila* homolog of the yeast chromatin remodeling factor SWI2/SNF2. Genetics *148*, 251–265.

Elrod-Erickson, M., Mishra, S., and Schneider, D. (2000). Interactions between the cellular and humoral immune responses in *Drosophila*. Curr. Biol. *10*, 781–784.

el Shatoury, H.H. (1955). The structure of the lymph glands of *Drosophila* larvae. Roux Arch. EntwMech. Organ. *147*, 489–495.

Fadok, V.A., Bratton, D.L., Rose, D.M., Pearson, A., Ezekewitz, R.A., and Henson, P.M. (2000). A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature *405*, 85–90.

Fehling, H.J., Gilfillan, S., and Ceredig, R. (1999). $\alpha\ \beta/\gamma\ \delta$ lineage commitment in the thymus of normal and genetically manipulated mice. Adv. Immunol. *71*, 1–76.

Ferrara, N., Gerber, H.P., and LeCouter, J. (2003). The biology of VEGF and its receptors. Nat. Med. *9*, 669–676.

Fessler, L.I., Nelson, R.E., and Fessler, J.H. (1994). *Drosophila* extracellular matrix. Methods Enzymol. *245*, 271–294.

Fisher, A.G. (2002). Cellular identity and lineage choice. Nat. Rev. Immunol. *2*, 977–982.

Flores, G.V., Daga, A., Kalhor, H.R., and Banerjee, U. (1998). Lozenge is expressed in pluripotent precursor cells and patterns multiple cell types in the *Drosophila* eye through the control of cell-specific transcription factors. Development *125*, 3681–3687.

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.

Fossett, N., Hyman, K., Gajewski, K., Orkin, S.H., and Schulz, R.A. (2003). Combinatorial interactions of serpent, lozenge, and U-shaped regulate crystal cell lineage commitment during *Drosophila* hematopoiesis. Proc. Natl. Acad. Sci. USA *100*, 11451–11456.

Franc, N.C., Dimarcq, J.L., Lagueux, M., Hoffmann, J., and Ezekowitz, R.A. (1996). Croquemort, a novel *Drosophila* hemocyte/macrophage receptor that recognizes apoptotic cells. Immunity *4*, 431–443.

Frasch, M. (1995). Induction of visceral and cardiac mesoderm by ectodermal Dpp in the early *Drosophila* embryo. Nature *374*, 464–467.

Freeman, M.R., Delrow, J., Kim, J., Johnson, E., and Doe, C.Q. (2003). Unwrapping glial biology: Gcm target genes regulating glial development, diversification, and function. Neuron *38*, 567–580.

Fujiwara, Y., Chang, A.N., Williams, A.M., and Orkin, S.H. (2003). Functional overlap of GATA-1 and GATA-2 in primitive hematopoietic development. Blood, in press. Published online September 22, 2003.

Gabrilovich, D.I., Chen, H.L., Girgis, K.R., Cunningham, H.T., Meny, G.M., Nadaf, S., Kavanaugh, D., and Carbone, D.P. (1996). Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat. Med. *2*, 1096–1103.

Garzino, V., Pereira, A., Laurenti, P., Graba, Y., Levis, R.W., Le Parco, Y., and Pradel, J. (1992). Cell lineage-specific expression of modulo, a dose-dependent modifier of variegation in *Drosophila*. EMBO J. *11*, 4471–4479.

Gateff, E. (1994). Tumor-suppressor genes, hematopoietic malignancies and other hematopoietic disorders of *Drosophila melanogaster*. Ann. N Y Acad. Sci. *712*, 260–279.

Gehring, W.J. (2002). The genetic control of eye development and its implications for the evolution of the various eye-types. Int. J. Dev. Biol. *46*, 65–73.

Georgopoulos, K. (2002). Haematopoietic cell-fate decisions, chromatin regulation and ikaros. Nat. Rev. Immunol. *2*, 162–174.

Gerber, H.P., Malik, A.K., Solar, G.P., Sherman, D., Liang, X.H., Meng, G., Hong, K., Marsters, J.C., and Ferrara, N. (2002). VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. Nature *417*, 954–958.

Gerhart, J., and Kirschner, M. (1997). Cells, Embryos and Evolution: Toward a Cellular and Developmental Understanding of Phenotypic Variation and Evolutionary Adaptability (Boston, MA: Blackwell Science).

Gerondakis, S., Grossmann, M., Nakamura, Y., Pohl, T., and Grumont, R. (1999). Genetic approaches in mice to understand Rel/NF-κB and IκB function: transgenics and knockouts. Oncogene *18*, 6888–6895.

Gerttula, S., Jin, Y.S., and Anderson, K.V. (1988). Zygotic expression and activity of the *Drosophila* Toll gene, a gene required maternally for embryonic dorsal-ventral pattern formation. Genetics *119*, 123–133.

Ghelelovitch, S. (1969). Melanotic tumors in *Drosophila melanogaster*. Natl. Cancer Inst. Monogr. *31*, 263–275.

Gisselbrecht, S., Skeath, J.B., Doe, C.Q., and Michelson, A.M. (1996). heartless encodes a fibroblast growth factor receptor (DFR1/DFGF-R2) involved in the directional migration of early mesodermal cells in the *Drosophila* embryo. Genes Dev. *10*, 3003–3017.

Godin, I., and Cumano, A. (2002). The hare and the tortoise: an embryonic haematopoietic race. Nat. Rev. Immunol. *2*, 593–604.

Govind, S. (1999). Control of development and immunity by rel transcription factors in *Drosophila*. Oncogene *18*, 6875–6887.

Gunther, T., Chen, Z.F., Kim, J., Priemel, M., Rueger, J.M., Amling, M., Moseley, J.M., Martin, T.J., Anderson, D.J., and Karsenty, G. (2000). Genetic ablation of parathyroid glands reveals another source of parathyroid hormone. Nature *406*, 199–203.

Gupta, A.P. (1979). Hemocyte types: their structures, synonymies, interrelationships, and taxonomic significance. In Insect Hemocyte, A.P. Gupta, ed. (Cambridge, UK: Cambridge University Press), pp. 85–127.

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.

Hanratty, W.P., and Dearolf, C.R. (1993). The *Drosophila Tumorous-lethal* hematopoietic oncogene is a dominant mutation in the *hopscotch* locus. Mol. Gen. Genet. *238*, 33–37.

Hanratty, W.P., and Ryerse, J.S. (1981). A genetic melanotic neoplasm of *Drosophila melanogaster*. Dev. Biol. *83*, 238–249.

Harrison, D.A., Binari, R., Nahreini, T.S., Gilman, M., and Perrimon, N. (1995). Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. EMBO J. *14*, 2857–2865.

Hashimoto, C., Hudson, K.L., and Anderson, K.V. (1988). The Toll gene of *Drosophila*, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell *52*, 269–279.

Hattori, K., Dias, S., Heissig, B., Hackett, N.R., Lyden, D., Tateno, M., Hicklin, D.J., Zhu, Z., Witte, L., Crystal, R.G., et al. (2001). Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. J. Exp. Med. *193*, 1005–1014.

Haughn, L., Hawley, R.G., Morrison, D.K., von Boehmer, H., and Hockenbery, D.M. (2003). BCL-2 and BCL-XL restrict lineage choice during hematopoietic differentiation. J. Biol. Chem. *278*, 25158–25165.

Heino, T.I., Karpanen, T., Wahlstrom, G., Pulkkinen, M., Eriksson, U., Alitalo, K., and Roos, C. (2001). The *Drosophila* VEGF receptor homolog is expressed in hemocytes. Mech. Dev. *109*, 69–77.

Herbomel, P., Thisse, B., and Thisse, C. (1999). Ontogeny and behaviour of early macrophages in the zebrafish embryo. Development *126*, 3735–3745.

Hess, J.L., Yu, B.D., Li, B., Hanson, R., and Korsmeyer, S.J. (1997). Defects in yolk sac hematopoiesis in Mll-null embryos. Blood *90*, 1799–1806.

Hetru, C., Troxler, L., and Hoffmann, J.A. (2003). *Drosophila melanogaster* antimicrobial defense. J. Infect. Dis. *187* (Suppl 2), S327–S334.

Hollande, A.C. (1909). Contribution a l'etude du sang des Coleopteres. Arch. Zool. Exp. Gen. (Ser 5) *21*, 271–294.

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

Holz, A., Bossinger, B., Strasser, T., Janning, W., and Klapper, R. (2003). The two origins of hemocytes in *Drosophila*. Development *130*, 4955–4962.

Hosoya, T., Takizawa, K., Nitta, K., and Hotta, Y. (1995). glial cells missing: a binary switch between neuronal and glial determination in *Drosophila*. Cell *82*, 1025–1036.

Hou, S.X., Zheng, Z., Chen, X., and Perrimon, N. (2002). The Jak/STAT pathway in model organisms: emerging roles in cell movement. Dev. Cell *3*, 765–778.

Ip, Y.T., and Levine, M. (1994). Molecular genetics of *Drosophila* immunity. Curr. Opin. Genet. Dev. *4*, 672–677.

Iritani, B.M., Forbush, K.A., Farrar, M.A., and Perlmutter, R.M. (1997). Control of B cell development by Ras-mediated activation of Raf. EMBO J. *16*, 7019–7031.

Jones, B.W., Fetter, R.D., Tear, G., and Goodman, C.S. (1995). glial cells missing: a genetic switch that controls glial versus neuronal fate. Cell *82*, 1013–1023.

Kaminker, J.S., Singh, R., Lebestky, T., Yan, H., and Banerjee, U. (2001). Redundant function of Runt Domain binding partners, Big brother and Brother, during *Drosophila* development. Development *128*, 2639–2648.

Kammerer, M., and Giangrande, A. (2001). Glide2, a second glial promoting factor in *Drosophila melanogaster*. EMBO J. *20*, 4664–4673.

Karanu, F.N., Murdoch, B., Gallacher, L., Wu, D.M., Koremoto, M., Sakano, S., and Bhatia, M. (2000). The notch ligand jagged-1 represents a novel growth factor of human hematopoietic stem cells. J. Exp. Med. *192*, 1365–1372.

Katz, S.G., Cantor, A.B., and Orkin, S.H. (2002). Interaction between FOG-1 and the corepressor C-terminal binding protein is dispensable for normal erythropoiesis in vivo. Mol. Cell. Biol. *22*, 3121–3128.

Kieslinger, M., Woldman, I., Moriggl, R., Hofmann, J., Marine, J.C., Ihle, J.N., Beug, H., and Decker, T. (2000). Antiapoptotic activity of Stat5 required during terminal stages of myeloid differentiation. Genes Dev. *14*, 232–244.

Kiger, J.A., Jr., Natzle, J.E., and Green, M.M. (2001). Hemocytes are essential for wing maturation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *98*, 10190–10195.

Kim, J., Jones, B.W., Zock, C., Chen, Z., Wang, H., Goodman, C.S., and Anderson, D.J. (1998). Isolation and characterization of mammalian homologs of the *Drosophila* gene glial cells missing. Proc. Natl. Acad. Sci. USA *95*, 12364–12369.

Kitabayashi, I., Yokoyama, A., Shimizu, K., and Ohki, M. (1998). Interaction and functional cooperation of the leukemia-associated factors AML1 and p300 in myeloid cell differentiation. EMBO J. *17*, 2994–3004.

Klapper, R., Holz, A., and Janning, W. (1998). Fate map and cell lineage relationships of thoracic and abdominal mesodermal anlagen in *Drosophila melanogaster*. Mech. Dev. *71*, 77–87.

Kmita, M., and Duboule, D. (2003). Organizing axes in time and space; 25 years of colinear tinkering. Science *301*, 331–333.

Kondo, M., Weissman, I.L., and Akashi, K. (1997). Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell *91*, 661–672.

Kondo, M., Wagers, A.J., Manz, M.G., Prohaska, S.S., Scherer, D.C., Beilhack, G.F., Shizuru, J.A., and Weissman, I.L. (2003). Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annu. Rev. Immunol. *21*, 759–806.

Kowenz-Leutz, E., and Leutz, A. (1999). A C/EBP β isoform recruits the SWI/SNF complex to activate myeloid genes. Mol. Cell *4*, 735–743.

Kumano, K., Chiba, S., Shimizu, K., Yamagata, T., Hosoya, N., Saito, T., Takahashi, T., Hamada, Y., and Hirai, H. (2001). Notch1 inhibits differentiation of hematopoietic cells by sustaining GATA-2 expression. Blood *98*, 3283–3289.

Kumano, K., Chiba, S., Kunisato, A., Sata, M., Saito, T., Nakagami-Yamaguchi, E., Yamaguchi, T., Masuda, S., Shimizu, K., Takahashi, T., et al. (2003). Notch1 but not Notch2 is essential for generating hematopoietic stem cells from endothelial cells. Immunity *18*, 699–711.

Kung, A.L., Rebel, V.I., Bronson, R.T., Ch'ng, L.E., Sieff, C.A., Livingston, D.M., and Yao, T.P. (2000). Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. Genes Dev. *14*, 272–277.

Kurdistani, S.K., and Grunstein, M. (2003). Histone acetylation and deacetylation in yeast. Nat. Rev. Mol. Cell Biol. *4*, 276–284.

Kurucz, E., Zettervall, C.J., Sinka, R., Vilmos, P., Pivarcsi, A., Ekengren, S., Hegedus, Z., Ando, I., and Hultmark, D. (2003). Hemese, a hemocyte-specific transmembrane protein, affects the cellular immune response in *Drosophila*. Proc. Natl. Acad. Sci. USA *100*, 2622–2627.

Kwon, E.J., Park, H.S., Kim, Y.S., Oh, E.J., Nishida, Y., Matsukage, A., Yoo, M.A., and Yamaguchi, M. (2000). Transcriptional regulation of the *Drosophila* raf proto-oncogene by *Drosophila* STAT during development and in immune response. J. Biol. Chem. *275*, 19824–19830.

Lacronique, V., Boureux, A., Valle, V.D., Poirel, H., Quang, C.T., Mauchauffe, M., Berthou, C., Lessard, M., Berger, R., Ghysdael, J., and Bernard, O.A. (1997). A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science *278*, 1309–1312.

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

Lebestky, T.J. (2001). Specification of hematopoietic lineage in *Drosophila melanogaster*. PhD thesis, University of California, Los Angeles.

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

Lebestky, T., Jung, S.H., and Banerjee, U. (2003). A Serrate-expressing signaling center controls *Drosophila* hematopoiesis. Genes Dev. *17*, 348–353.

Lee, C.H., Murphy, M.R., Lee, J.S., and Chung, J.H. (1999). Targeting a SWI/SNF-related chromatin remodeling complex to the β-globin promoter in erythroid cells. Proc. Natl. Acad. Sci. USA *96*, 12311–12315.

Lemaitre, B., Meister, M., Govind, S., Georgel, P., Steward, R., Reichhart, J.M., and Hoffmann, J.A. (1995). Functional analysis and regulation of nuclear import of dorsal during the immune response in *Drosophila*. EMBO J. *14*, 536–545.

Lessard, J., and Sauvageau, G. (2003). Polycomb group genes as epigenetic regulators of normal and leukemic hemopoiesis. Exp. Hematol. *31*, 567–585.

Letting, D.L., Rakowski, C., Weiss, M.J., and Blobel, G.A. (2003). Formation of a tissue-specific histone acetylation pattern by the hematopoietic transcription factor GATA-1. Mol. Cell. Biol. *23*, 1334–1340.

Levanon, D., Goldstein, R.E., Bernstein, Y., Tang, H., Goldenberg, D., Stifani, S., Paroush, Z., and Groner, Y. (1998). Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. Proc. Natl. Acad. Sci. USA *95*, 11590–11595.

Li, L.H., and Gergen, J.P. (1999). Differential interactions between Brother proteins and Runt domain proteins in the *Drosophila* embryo and eye. Development *126*, 3313–3322.

Ligoxygakis, P., Pelte, N., Ji, C., Leclerc, V., Duvic, B., Belvin, M., Jiang, H., Hoffmann, J.A., and Reichhart, J.M. (2002). A serpin mutant links Toll activation to melanization in the host defence of *Drosophila*. EMBO J. *21*, 6330–6337.

Lindsell, C.E., Shawber, C.J., Boulter, J., and Weinmaster, G. (1995). Jagged: a mammalian ligand that activates Notch1. Cell *80*, 909–917.

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.

Luo, H., Hanratty, W.P., and Dearolf, C.R. (1995). An amino acid substitution in the *Drosophila* hopTum-l Jak kinase causes leukemia-like hematopoietic defects. EMBO J. *14*, 1412–1420.

Luo, H., Rose, P.E., Roberts, T.M., and Dearolf, C.R. (2002). The Hopscotch Jak kinase requires the Raf pathway to promote blood cell activation and differentiation in *Drosophila*. Mol. Genet. Genomics *267*, 57–63.

Lutterbach, B., and Hiebert, S.W. (2000). Role of the transcription factor AML-1 in acute leukemia and hematopoietic differentiation. Gene *245*, 223–235.

Maduro, M.F., and Rothman, J.H. (2002). Making worm guts: the gene regulatory network of the *Caenorhabditis elegans* endoderm. Dev. Biol. *246*, 68–85.

Martin, G. (2001). Making a vertebrate limb: new players enter from the wings. Bioessays *23*, 865–868.

Mikula, M., Schreiber, M., Husak, Z., Kucerova, L., Ruth, J., Wieser, R., Zatloukal, K., Beug, H., Wagner, E.F., and Baccarini, M. (2001). Embryonic lethality and fetal liver apoptosis in mice lacking the c-raf-1 gene. EMBO J. *20*, 1952–1962.

Miller, R.W., and Rubinstein, J.H. (1995). Tumors in Rubinstein-Taybi syndrome. Am. J. Med. Genet. *56*, 112–115.

Munier, A.I., Doucet, D., Perrodou, E., Zachary, D., Meister, M., Hoffmann, J.A., Janeway, C.A., Jr., and Lagueux, M. (2002). PVF2, a PDGF/VEGF-like growth factor, induces hemocyte proliferation in *Drosophila* larvae. EMBO Rep. *3*, 1195–1200.

Murray, M.A., Fessler, L.I., and Palka, J. (1995). Changing distributions of extracellular matrix components during early wing morphogenesis in *Drosophila*. Dev. Biol. *168*, 150–165.

Nappi, A.J. (1975). Inhibition by parasites of melanotic tumour formation in *Drosophila melanogaster*. Nature *255*, 402–404.

Nappi, A.J., and Vass, E. (1998). Hydrogen peroxide production in immune-reactive *Drosophila melanogaster*. J. Parasitol. *84*, 1150–1157.

Nappi, A.J., Vass, E., Frey, F., and Carton, Y. (2000). Nitric oxide involvement in *Drosophila* immunity. Nitric Oxide *4*, 423–430.

Neubauer, H., Cumano, A., Muller, M., Wu, H., Huffstadt, U., and Pfeffer, K. (1998). Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell *93*, 397–409.

Ogawa, H., Ueda, T., Aoyama, T., Aronheim, A., Nagata, S., and Fukunaga, R. (2003). A SWI2/SNF2-type ATPase/helicase protein, mDomino, interacts with myeloid zinc finger protein 2A (MZF-2A) to regulate its transcriptional activity. Genes Cells *8*, 325–339.

Ohinata, H., Tochinai, S., and Katagiri, C. (1990). Occurrence of nonlymphoid leukocytes that are not derived from blood islands in *Xenopus laevis* larvae. Dev. Biol. *141*, 123–129.

Okuda, T., van Deursen, J., Hiebert, S.W., Grosveld, G., and Downing, J.R. (1996). AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell *84*, 321–330.

Orkin, S.H., Shivdasani, R.A., Fujiwara, Y., and McDevitt, M.A. (1998). Transcription factor GATA-1 in megakaryocyte development. Stem Cells *16* (Suppl 2), 79–83.

Parganas, E., Wang, D., Stravopodis, D., Topham, D.J., Marine, J.C., Teglund, S., Vanin, E.F., Bodner, S., Colamonici, O.R., van Deursen, J.M., et al. (1998). Jak2 is essential for signaling through a variety of cytokine receptors. Cell *93*, 385–395.

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

Peeples, E.E., Geisler, A., Whitcraft, C.J., and Oliver, C. (1969). Comparative studies of phenol oxidase activity during pupal development of three lozenge mutants (lzs, lz, lzk) of *Drosophila melanogaster*. Genetics *62*, 161–170.

Peeters, P., Raynaud, S.D., Cools, J., Wlodarska, I., Grosgeorge, J., Philip, P., Monpoux, F., Van Rompaey, L., Baens, M., Van den Berghe, H., and Marynen, P. (1997). Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. Blood *90*, 2535–2540.

Perrimon, N., and Mahowald, A.P. (1986). l(1)hopscotch, a larval-pupal zygotic lethal with a specific maternal effect on segmentation in *Drosophila*. Dev. Biol. *118*, 28–41.

Petrij, F., Giles, R.H., Dauwerse, H.G., Saris, J.J., Hennekam, R.C., Masuno, M., Tommerup, N., van Ommen, G.J., Goodman, R.H., Peters, D.J., et al. (1995). Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature *376*, 348–351.

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

Pui, J.C., Allman, D., Xu, L., DeRocco, S., Karnell, F.G., Bakkour, S., Lee, J.Y., Kadesch, T., Hardy, R.R., Aster, J.C., and Pear, W.S. (1999). Notch1 expression in early lymphopoiesis influences B versus T lineage determination. Immunity *11*, 299–308.

Qiu, P., Pan, P.C., and Govind, S. (1998). A role for the *Drosophila* Toll/Cactus pathway in larval hematopoiesis. Development *125*, 1909–1920.

Radtke, F., Wilson, A., Stark, G., Bauer, M., van Meerwijk, J., MacDonald, H.R., and Aguet, M. (1999). Deficient T cell fate specification in mice with an induced inactivation of Notch1. Immunity *10*, 547–558.

Radtke, F., Wilson, A., Ernst, B., and MacDonald, H.R. (2002). The role of Notch signaling during hematopoietic lineage commitment. Immunol. Rev. *187*, 65–74.

Ramet, M., Pearson, A., Manfruelli, P., Li, X., Koziel, H., Gobel, V., Chung, E., Krieger, M., and Ezekowitz, R.A. (2001). *Drosophila* scavenger receptor CI is a pattern recognition receptor for bacteria. Immunity *15*, 1027–1038.

Ramet, M., Lanot, R., Zachary, D., and Manfruelli, P. (2002a). JNK signaling pathway is required for efficient wound healing in *Drosophila*. Dev. Biol. *241*, 145–156.

Ramet, M., Manfruelli, P., Pearson, A., Mathey-Prevot, B., and Ezekowitz, R.A. (2002b). Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli*. Nature *416*, 644–648.

Rane, S.G., and Reddy, E.P. (2002). JAKs, STATs and Src kinases in hematopoiesis. Oncogene *21*, 3334–3358.

Ratcliffe, N.A., and Rowley, A.F. (1979). Role of hemocytes in defense against biological agents. In Insect Hemocytes, A.P. Gupta, ed. (Cambridge, UK: Cambridge University Press), pp. 331–414.

Rayet, B., and Gelinas, C. (1999). Aberrant rel/nfkb genes and activity in human cancer. Oncogene *18*, 6938–6947.

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.

Remillieux-Leschelle, N., Santamaria, P., and Randsholt, N.B. (2002). Regulation of larval hematopoiesis in *Drosophila melanogaster*: a role for the multi sex combs gene. Genetics *162*, 1259–1274.

Rizki, T.M. (1956). Blood cells of *Drosophila* as related to metamorphosis. In Physiology of Insect Development, F.L. Campbell, ed. (Chicago, IL: Chicago University Press), pp. 91–94.

Rizki, T.M. (1962). Experimental analysis of hemocyte morphology in insects. Am. Zool. *2*, 247–256.

Rizki, T.M. (1978). The circulatory system and associated cells and tissues. In The Genetics and Biology of *Drosophila*, M. Ashburner and T.R.F. Wright, eds. (New York, London: Academic Press).

Rizki, R.M., and Rizki, T.M. (1974). Basement membrane abnormalities in melanotic tumor formation of *Drosophila*. Experientia *30*, 543–546.

Rizki, R.M., and Rizki, T.M. (1979). Cell interactions in the differentiation of a melanotic tumor in *Drosophila*. Differentiation *12*, 167–178.

Rizki, T.M., and Rizki, R.M. (1980). Properties of the larval hemocytes of *Drosophila melanogaster*. Experientia *36*, 1223–1226.

Rizki, T.M., and Rizki, R.M. (1981). Alleles of lz as suppressors of the Bc-phene in *Drosophila melanogaster*. Genetics *97*, S90.

Rizki, T.M., and Rizki, R.M. (1985). Paracrystalline inclusions of *D. melanogaster* hemocytes have prophenoloxidases. Genetics *110*, S98.

Rizki, T.M., and Rizki, R.M. (1992). Lamellocyte differentiation in *Drosophila* larvae parasitized by *Leptopilina*. Dev. Comp. Immunol. *16*, 103–110.

Rizki, T.M., Rizki, R.M., and Grell, E.H. (1980). A mutant affecting the crystal cells in *Drosophila melanogaster*. Rouxs Arch. Dev. Biol. *188*, 91–99.

Robertson, C.W. (1936). The metamorphosis of *Drosophila melanogaster*, including an accurately timed account of the principal morphological changes. J. Morphol. *59*, 351–399.

Robey, E., Chang, D., Itano, A., Cado, D., Alexander, H., Lans, D., Weinmaster, G., and Salmon, P. (1996). An activated form of Notch influences the choice between CD4 and CD8 T cell lineages. Cell *87*, 483–492.

Roos, E., Bjorklund, G., and Engstrom, Y. (1998). In vivo regulation of tissue-specific and LPS-inducible expression of the *Drosophila* Cecropin genes. Insect Mol. Biol. *7*, 51–62.

Rosetto, M., Engstrom, Y., Baldari, C.T., Telford, J.L., and Hultmark, D. (1995). Signals from the IL-1 receptor homolog, Toll, can activate an immune response in a *Drosophila* hemocyte cell line. Biochem. Biophys. Res. Commun. *209*, 111–116.

Rugendorff, A.E., Younossi-Hartenstein, A., and Hartenstein, V. (1994). Embryonic origin and differentiation of the *Drosophila* heart. Rouxs Arch. Dev. Biol. *203*, 266–280.

Ruhf, M.L., Braun, A., Papoulas, O., Tamkun, J.W., Randsholt, N., and Meister, M. (2001). The domino gene of *Drosophila* encodes novel members of the SWI2/SNF2 family of DNA-dependent ATPases, which contribute to the silencing of homeotic genes. Development *128*, 1429–1441.

Russo, J., Dupas, S., Frey, F., Carton, Y., and Brehelin, M. (1996). Insect immunity: early events in the encapsulation process of parasitoid (*Leptopilina boulardi*) eggs in resistant and susceptible strains of *Drosophila*. Parasitology *112*, 135–142.

Saito, T., Chiba, S., Ichikawa, M., Kunisato, A., Asai, T., Shimizu, K., Yamaguchi, T., Yamamoto, G., Seo, S., Kumano, K., et al. (2003). Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. Immunity *18*, 675–685.

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.

Samakovlis, C., Kimbrell, D.A., Kylsten, P., Engstrom, A., and Hultmark, D. (1990). The immune response in *Drosophila*: pattern of cecropin expression and biological activity. EMBO J. *9*, 2969–2976.

Sasaki, K., Yagi, H., Bronson, R.T., Tominaga, K., Matsunashi, T., Deguchi, K., Tani, Y., Kishimoto, T., and Komori, T. (1996). Absence of fetal liver hematopoiesis in mice deficient in transcriptional coactivator core binding factor β. Proc. Natl. Acad. Sci. USA *93*, 12359–12363.

Schneider, D.S., Hudson, K.L., Lin, T.Y., and Anderson, K.V. (1991). Dominant and recessive mutations define functional domains of Toll, a transmembrane protein required for dorsal-ventral polarity in the *Drosophila* embryo. Genes Dev. *5*, 797–807.

Schreiber, J., Riethmacher-Sonnenberg, E., Riethmacher, D., Tuerk, E.E., Enderich, J., Bosl, M.R., and Wegner, M. (2000). Placental

failure in mice lacking the mammalian homolog of glial cells missing, GCMa. Mol. Cell. Biol. *20*, 2466–2474.

Schwaller, J., Parganas, E., Wang, D., Cain, D., Aster, J.C., Williams, I.R., Lee, C.K., Gerthner, R., Kitamura, T., Frantsve, J., et al. (2000). Stat5 is essential for the myelo- and lymphoproliferative disease induced by TEL/JAK2. Mol. Cell *6*, 693–704.

Sears, H.C., Kennedy, C.J., and Garrity, P.A. (2003). Macrophage-mediated corpse engulfment is required for normal *Drosophila* CNS morphogenesis. Development *130*, 3557–3565.

Shivdasani, R.A., Fujiwara, Y., McDevitt, M.A., and Orkin, S.H. (1997). A lineage-selective knockout establishes the critical role of transcription factor GATA-1 in megakaryocyte growth and platelet development. EMBO J. *16*, 3965–3973.

Shrestha, R., and Gateff, E. (1982). Ultrastructure and cytochemistry of the cell types in the larval hematopoietic organs and hemolymph of *Drosophila melanogaster*. Dev. Growth Differ. *24*, 65–82.

Siddall, N.A., Behan, K.J., Crew, J.R., Cheung, T.L., Fair, J.A., Batterham, P., and Pollock, J.A. (2003). Mutations in lozenge and D-Pax2 invoke ectopic patterned cell death in the developing *Drosophila* eye using distinct mechanisms. Dev. Genes Evol. *213*, 107–119.

Silverman, N., and Maniatis, T. (2001). NF-κB signaling pathways in mammalian and insect innate immunity. Genes Dev. *15*, 2321–2342.

Silvers, M., and Hanratty, W.P. (1984). Alterations in the production of hemocytes due to a neoplastic mutation of *Drosophila melanogaster*. J. Invertebr. Pathol. *44*, 324–328.

Soderhall, K., and Cerenius, L. (1998). Role of the prophenoloxidase-activating system in invertebrate immunity. Curr. Opin. Immunol. *10*, 23–28.

Sorrentino, R.P., Carton, Y., and Govind, S. (2002). Cellular immune response to parasite infection in the *Drosophila* lymph gland is developmentally regulated. Dev. Biol. *243*, 65–80.

Speck, N.A., and Gilliland, D.G. (2002). Core-binding factors in haematopoiesis and leukaemia. Nat. Rev. Cancer *2*, 502–513.

Stark, M.B., and Marshall, A.K. (1930). The blood-forming organ of the larva of *Drosophila melanogaster*. Bell Telephone System Tech. Pub. Monogr. *1931*, 1204–1206.

Struhl, K. (1998). Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. *12*, 599–606.

Sundqvist, A., Sollerbrant, K., and Svensson, C. (1998). The carboxy-terminal region of adenovirus E1A activates transcription through targeting of a C-terminal binding protein-histone deacetylase complex. FEBS Lett. *429*, 183–188.

Svensson, E.C., Tufts, R.L., Polk, C.E., and Leiden, J.M. (1999). Molecular cloning of FOG-2: a modulator of transcription factor GATA-4 in cardiomyocytes. Proc. Natl. Acad. Sci. USA *96*, 956–961.

Takahashi, K., and Naito, M. (1993). Development, differentiation, and proliferation of macrophages in the rat yolk sac. Tissue Cell *25*, 351–362.

Takahashi, K., Yamamura, F., and Naito, M. (1989). Differentiation, maturation, and proliferation of macrophages in the mouse yolk sac: a light-microscopic, enzyme-cytochemical, immunohistochemical, and ultrastructural study. J. Leukoc. Biol. *45*, 87–96.

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.

Ting, C.N., Olson, M.C., Barton, K.P., and Leiden, J.M. (1996). Transcription factor GATA-3 is required for development of the T-cell lineage. Nature *384*, 474–478.

Traver, D., and Zon, L.I. (2002). Walking the walk: migration and other common themes in blood and vascular development. Cell *108*, 731–734.

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

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.

Turner, J., and Crossley, M. (1998). Cloning and characterization of mCtBP2, a co-repressor that associates with basic Kruppel-like factor and other mammalian transcriptional regulators. EMBO J. *17*, 5129–5140.

Tzou, P., De Gregorio, E., and Lemaitre, B. (2002). How *Drosophila* combats microbial infection: a model to study innate immunity and host-pathogen interactions. Curr. Opin. Microbiol. *5*, 102–110.

Varnum-Finney, B., Purton, L.E., Yu, M., Brashem-Stein, C., Flowers, D., Staats, S., Moore, K.A., Le Roux, I., Mann, R., Gray, G., et al. (1998). The Notch ligand, Jagged-1, influences the development of primitive hematopoietic precursor cells. Blood *91*, 4084–4091.

Vincent, S., Vonesch, J.L., and Giangrande, A. (1996). Glide directs glial fate commitment and cell fate switch between neurones and glia. Development *122*, 131–139.

Walker, L., Carlson, A., Tan-Pertel, H.T., Weinmaster, G., and Gasson, J. (2001). The notch receptor and its ligands are selectively expressed during hematopoietic development in the mouse. Stem Cells *19*, 543–552.

Waltzer, L., Bataille, L., Peyrefitte, S., and Haenlin, M. (2002). Two isoforms of Serpent containing either one or two GATA zinc fingers have different roles in *Drosophila* haematopoiesis. EMBO J. *21*, 5477–5486.

Wang, Q., Stacy, T., Binder, M., Marin-Padilla, M., Sharpe, A.H., and Speck, N.A. (1996a). Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. Proc. Natl. Acad. Sci. USA *93*, 3444–3449.

Wang, Q., Stacy, T., Miller, J.D., Lewis, A.F., Gu, T.L., Huang, X., Bushweller, J.H., Bories, J.C., Alt, F.W., Ryan, G., et al. (1996b). The CBFβ subunit is essential for CBFα2 (AML1) function in vivo. Cell *87*, 697–708.

Washburn, T., Schweighoffer, E., Gridley, T., Chang, D., Fowlkes, B.J., Cado, D., and Robey, E. (1997). Notch activity influences the αβ versus γδ T cell lineage decision. Cell *88*, 833–843.

Wegner, M., and Riethmacher, D. (2001). Chronicles of a switch hunt: gcm genes in development. Trends Genet. *17*, 286–290.

Wigglesworth, V.B. (1939). The Principles of Insect Physiology, First Edition (Methuen, London).

Wilson, A., MacDonald, H.R., and Radtke, F. (2001). Notch 1-deficient common lymphoid precursors adopt a B cell fate in the thymus. J. Exp. Med. *194*, 1003–1012.

Wu, X., Golden, K., and Bodmer, R. (1995). Heart development in *Drosophila* requires the segment polarity gene wingless. Dev. Biol. *169*, 619–628.

Yagi, H., Deguchi, K., Aono, A., Tani, Y., Kishimoto, T., and Komori, T. (1998). Growth disturbance in fetal liver hematopoiesis of Mll-mutant mice. Blood *92*, 108–117.

Yasothornsrikul, S., Davis, W.J., Cramer, G., Kimbrell, D.A., and Dearolf, C.R. (1997). viking: identification and characterization of a second type IV collagen in *Drosophila*. Gene *198*, 17–25.

Yeager, J.F. (1945). The blood picture of the southern armyworm (*Prodenia eridania*). J. Agric. Res. *71*, 1–40.

Zhang, W., Kadam, S., Emerson, B.M., and Bieker, J.J. (2001). Site-specific acetylation by p300 or CREB binding protein regulates erythroid Kruppel-like factor transcriptional activity via its interaction with the SWI-SNF complex. Mol. Cell. Biol. *21*, 2413–2422.

Zhou, Z., Hartwieg, E., and Horvitz, H.R. (2001). CED-1 is a transmembrane receptor that mediates cell corpse engulfment in *C. elegans*. Cell *104*, 43–56.