Regulation of Larval Hematopoiesis in *Drosophila melanogaster*: A Role for the *multi sex combs* Gene

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ABSTRACT

Drosophila larval hematopoietic organs produce circulating hemocytes that ensure the cellular host defense by recognizing and neutralizing non-self or noxious objects through phagocytosis or encapsulation and melanization. Hematopoietic lineage specification as well as blood cell proliferation and differentiation are tightly controlled. Mutations in genes that regulate lymph gland cell proliferation and hemocyte numbers in the body cavity cause hematopoietic organ overgrowth and hemocyte overproliferation. Occasionally, mutant hemocytes invade self-tissues, behaving like neoplastic malignant cells. Two alleles of the Polycomb group (PcG) gene *multi sex combs* (*mxc*) were previously isolated as such lethal malignant blood neoplasms. PcG genes regulate *Hox* gene expression in vertebrates and invertebrates and participate in mammalian hematopoiesis control. Hence we investigated the need for *mxc* in Drosophila hematopoietic organs and circulating hemocytes. We show that *mxc*-induced hematopoietic hyperplasia is cell autonomous and that *mxc* mainly controls plasmatocyte lineage proliferation and differentiation in lymph glands and circulating hemocytes. Loss of the Toll pathway, which plays a similar role in hematopoiesis, counteracted *mxc* hemocyte proliferation but not *mxc* hemocyte differentiation. Several PcG genes tested in trans had no effects on *mxc* hematopoietic phenotypes, whereas the *trithorax* group gene brahma is important for normal and mutant hematopoiesis control. We propose that *mxc* provides one of the regulatory inputs in larval hematopoiesis that control normal rates of plasmatocyte and crystal lineage proliferation as well as normal rates and timing of hemocyte differentiation.

DROSOPHILA larval circulating blood cells carry out the cellular host defense response through phagocytosis or encapsulation and melanization. Most wild-type circulating hemocytes belong to a monocyte-like phagocytic cell lineage (reviewed in Dearolf 1998; Fossett and Schulz 2001). They are called plasmatocytes, podocytes, or macrophages according to their shape, their adhesion properties, and the stage of development (Gateff 1978; Rizki 1978; Lanot et al. 2001; Meister and Govind 2002). Phagocytic cells eliminate small foreign objects and, in pupae, the lysing larval tissues. Less than 1% of circulating hemocytes are flat lamellocytes that encapsulate larger “non-self” objects (Rizki 1978; Rizki and Rizki 1980). Crystal cells represent a second hematopoietic cell lineage (Dearolf 1998; Fossett and Schulz 2001), which is required for melanization of lamellocytes after encapsulation (Rizki 1978). Specification of hemocyte progenitor cells and regulation of lineage commitment and differentiation depend on a series of evolutionarily conserved transcription factors (Lebestky et al. 2000; reviewed in Fossett and Schulz 2001). Drosophila blood cells are produced during two successive waves of hematopoiesis in embryos and larvae. All larval hemocytes are produced by the hematopoietic organs or lymph glands. This structure differentiates during late embryogenesis as two lobes along the anterior part of the dorsal vessel (Rugen dorff et al. 1994) and has in third instar larvae four to seven lobe pairs (Gateff 1978; Rizki 1978). Large anterior lobes contain most types of circulating hemocytes, whereas smaller posterior ones contain undifferentiated blast cells that normally give rise to macrophages at pupariation (Lanot et al. 2001). Lymph gland overgrowth, hemocyte proliferation, and hemocyte differentiation are inducible by immune challenge or by external aggression such as infestation by a parasitoid wasp, but they are also observed in certain mutant contexts (Watson et al. 1991; Rizki and Rizki 1992; reviewed in Dearolf 1998; Lanot et al. 2001). The corresponding larval phenotypes include hypertrophied hematopoietic organs, increased numbers of circulating hemocytes, and abnormal differentiation of lamellocytes that represent up to 50% of the cells. Melanotic masses often occur. They are formed by lamellocyte-covered capsules that contain melanized self-tissue (pseudotumors; Sparrow 1978; Watson et al. 1991; Dearolf 1998).

Such abnormal immune response phenotypes are caused by a number of mutations (Watson et al. 1991; Torok et al. 1993; Dearolf 1998), making these loci potential candidates for genes directly regulating hema-
PcG genes form a conserved group that collectively maintain expression patterns of important selector genes in vertebrates and invertebrates (reviewed in Pirrotta 1998; van Lohuizen 1999; Gebuhr et al. 2000; Brock and van Lohuizen 2001; Simon and Tamkun 2002). First isolated in Drosophila as negative trans-regulators of the *Hox* genes, PcG genes have been shown to act in conjunction with *trithorax* group (trxG) genes to maintain transcriptional regulation and provide a cellular memory mechanism throughout development, probably by changes in chromatin structure. Several mammalian PcG and trxG members are involved in hematopoiesis control (reviewed in van Lohuizen 1999; Gebuhr et al. 2000; Takihara and Hara 2000; Raaphorst et al. 2001). PcG genes show stage-specific expression differences in human bone marrow cells (Lessard et al. 1998). The mouse PcG gene *embryonic ectoderm development* (*eed*) negatively regulates myeloid and lymphoid progenitor cell proliferation in bone marrow (Lessard et al. 1999), whereas targeted disruption of murine PcG genes Bmi-1, Mel 18, Rae23/Mph1, and M33 all lead to loss or hypoproliferation of various hematopoietic tissues. The human *trithorax* homolog MLL is often affected in translocations associated with acute myeloid or lymphoblastic leukemias (reviewed in van Lohuizen 1999; Muller and Leutz 2001), and MLL−/+ mice present severe hematopoietic abnormalities (Yu et al. 1995). Furthermore, mammalian SWI/SNF chromatin remodeling proteins, which are homologs of the Drosophila trxG protein Brahma (BRM), have been implicated as important cofactors in the regulation of myeloid and erythroid genes (reviewed in Gebuhr et al. 2000; Muller and Leutz 2001). Little is known about possible roles of PcG and trxG genes in Drosophila hemopoiesis. The *domino* (*dom*) gene encodes SWI/SNF family DNA-dependent ATPases that interact with PcG products in negative homeotic gene regulation, and *dom* mutations induce hematopoietic disorders. Lymph glands show proliferation defects and the rare *dom* hemocytes that differentiate cannot cross the lymph gland basement membrane (Braun et al. 1997, 1998; Ruhe et al. 2001). *mxc* is the only Drosophila gene known to cause both abnormal hematopoiesis and homeotic transformations due to HOM/Hox gene gain of function (Saget et al. 1998). In view of this, we decided to analyze how regulation of larval hematopoiesis and of circulating hemocyte density were affected by *mxc* mutations, alone or in other mutant contexts that also control these processes.

Two signal transduction pathways are well characterized as controlling hematopoietic gain of function and steady-state numbers of hemocytes in Drosophila larvae. The conserved Toll/cactus/Rel/ NF-κB signaling pathway is one (Qu et al. 1998; reviewed in Dearolf 1998; Mathey-Prevot and Perrimon 1998; Meister and Govind 2002). Toll pathway activation leads to nuclear translocation of Rel/NF-κB transcription factors that regulate hematocyte division and differentiation. Enhanced Toll signaling induces hematopoietic organ hyperplasia, increases in circulating hemocytes, abnormal lamellocyte differentiation, and pseudotumors, whereas larvae with reduced Toll signaling have fewer hemocytes. Toll signaling and Rel/NF-κB proteins also control Drosophila humoral host defense (Govind 1999), and homologous vertebrate Toll/cactus-IκB/Rel pathways ensure similar functions (Qu et al. 1998; Grossmann et al. 1999).


Here, we describe hematopoietic phenotypes of several increasingly severe *mxc* alleles and confirm that *mxc* directly controls hematopoiesis. Development of plasmacyte and crystal cell lineages is affected by loss of *mxc*. We compared hematopoietic defects due to mutations of *mxc*, of the Toll, or of the JAK/STAT pathway and analyzed epistatic relations between *mxc* and these mutants. Loss of Toll signal is epistatic to loss of *mxc*. Diminished *D-stat* activity had no effect on *mxc* hematopoietic phenotypes whereas it partially rescued lamellocyte differentiation of Toll gain of function and both hematocyte overproliferation and differentiation induced by constitutive JAK activation. Any genetic combination of these proliferation activator contexts caused extreme lymph gland overgrowth together with reductions in circulating hemocyte numbers. Finally, we found that proliferation control by *mxc* is less dependent on other PcG genes than is segmental identity control, whereas the *trithorax* group gene *brahma* is important for normal...
and mutant hematopoiesis. We propose that mxc provides a hematopoiesis regulatory input that controls normal plasmacyte and crystal cell lineage development as well as normal rates and timing of hemocyte differentiation.

### MATERIALS AND METHODS

#### Fly strains and culture
Flies were grown on standard culture medium at 25°C, unless otherwise stated. mxc alleles have been described (Santamaria and Randsholt 1995; Docquier et al. 1996). Dorothy (Dor) encodes an ecysderoid UDP-glucosyl/UDP glucuronosyl transferase that is expressed in pericardial and lymph gland cells; the Dot-LacZ strain was a gift from D. Kimbrell (Rodriguez et al. 1996). Reporter line l(2)113/28 (from M. Meister) expressing β-galactosidase in lamellocytes is described by Braun et al. (1997). The ubiquitously β-galactosidase-expressing strain hsps83-LacZ contains several P(hsp83-LacZ) inserts. The β-galactosidase-free strain βgalw was provided by A. Shearn (Woodhouse et al. 1998). hopw/FM7 act-GFP flies were from M. Lagueux. Other Toll and JAK/STAT mutant strains were gifts from C. Dearolf or B. Lemaire. Mutant domino (dom) phenotypes are described by Ruf et al. (2001); dom strains were provided by M. Meister. Balanced chromosomes and all other mutants, including Black cells (Bc), tube (tub), Toll (Tl), cactus (cact), hopscotch (hop), D-stat, trxG, and PcG genes are described in FlyBase (1999).

#### Hemocyte counts
Wandering third instar larvae were bled in 5 μl of Drosophila Ringer on a hemocytometer. Mean hemocyte numbers per milliliter of hemolymph were estimated by counting the number of cells in a given surface area under a dissecting microscope. Each experiment involved an internal, nonmutant control category provided by sibling larvae issued from the same cross. Control larvae were collected from the same vials as experimental larvae and counted under similar conditions (see table legends for controls in each experiment). Hemocytes from at least 10 larvae were counted per genotype and most experiments were repeated. Genotypes were recognized by mouth-hook color, body shape, or the presence of actin- or histone3-driven GFP. mxc mutant chromosomes carry y and are maintained over Binsn. Phagocytic cells were recognized by their ability to absorb particles of India ink injected 2 hr previously into the larva (Lanot et al. 2001). Lamellocytes were identified by β-galactosidase expression, using the lamellocytes LacZreporter l(2)113/28 (Braun et al. 1997). Crystal cells were counted per larva; they were visualized by heating larvae for 10 min at 70°C in a water bath. Blackened cells were counted under a dissecting microscope. Hemocyte numbers were compared by Student’s t-tests. Blood cell type distributions and numbers of dividing cells were compared using chi-square tests.

#### Lymph gland transplantations
y mxc/Binsn females were crossed to homozygous hsps83-LacZ males at 25°C. Lymph glands from wandering third instar progeny, [yellow] for experimental and male [yellow] for control, were dissected in Drosophila Ringer and injected into the abdomen of 2- to 5-day-old βgalw/βgalw females, using a Drummond nanject automatic injector (Drummond Scientific, Broomall, PA). Injected females were grown overnight at 20°C, followed by 5 days at 25°C for proliferation tests and 3 weeks for survival tests. Proliferation test females were then dissected and X-Gal stained without prior fixation for 1–3 hr at 37°C. Stained tissues were fixed for 1–3 hr at 37°C and stained again for 15 min at 37°C. Stained tissues were fixed in 5

#### RESULTS
mxc mutations cause lymph gland overgrowth and hemocyte overproliferation: Two early pupal lethal mxc/ l(1)mbm mutants were isolated as showing severe hematopoietic neoplasia (Gateff 1978; Shrestha and Gateff 1982; Gateff and Mechler 1989). To determine whether such defects were particular for these alleles, we examined lymph glands of four hypomorphic mxc mutants with increasingly severe homeotic and developmental phenotypes that range from viable to larval le-
th. Their characteristics are summarized in Table 1. mxc mutant larvae develop pseudotumors mainly when raised under crowded conditions. In that case, <10% of mxc wild-type larvae exhibit pseudotumors while up to 25% older mxc siblings and most older mxc larvae do (Sparrow 1978; Saget et al. 1998). Effects of mxc on lymph glands were observed in X-Gal-stained mxc/Y;Dot-LacZ/+ late third instar larvae, compared to Binsn/Y;Dot-LacZ/+ siblings (Rodriguez et al. 1996). All four mutants exhibit overgrown lymph glands (Table 1; Figure 1). In mxc/Y, only some second and third lymph gland lobes showed hypertrophy, whereas more severe alleles induced stronger overgrowth in more posterior lobe pairs. To determine whether overgrowth was associated with increased cell divisions, we stained lymph glands with anti-phosphohistone H3 antibody, which labels cells undergoing mitosis. Lymph gland lobes from all mxc mutants exhibit increased mitotic activity as compared to wild type (Figure 2).

We examined numbers and relative proportions of circulating hemocytes in mxc larvae (Table 1; Figure 3). Numbers and ratios of plasmatocytes or macrophages, lamellocytes, and crystal cells in wild type have been described (Rizki 1978; Lanot et al. 2001; see Introduction). All control larvae showed mean hemocyte concentrations per milliliter that were within the range of previously published values for animals of comparable age (Gateff 1978; Breihelin 1982; Silvers and Hanratty 1984; Luo et al. 1995, 1997; Table 1). The same was true

### Table 1

<table>
<thead>
<tr>
<th>Genotype (N)</th>
<th>Phenotype</th>
<th>Lymph glands</th>
<th>Hemocytes (10^6)</th>
<th>Plasmatocytes (%)</th>
<th>Podocytes (%)</th>
<th>Lamellocytes (%)</th>
<th>Crystal cells per larva (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>y¹ ac¹ z¹ (10)</td>
<td>Viable; HT</td>
<td></td>
<td>1.9 ± 1.2</td>
<td>&gt;99</td>
<td>0.2</td>
<td>0.2</td>
<td>103 ± 80 (25)</td>
</tr>
<tr>
<td>Binsn/Y (35)</td>
<td></td>
<td></td>
<td>1.2 ± 0.5</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>0.2</td>
<td>133 ± 93 (16)</td>
</tr>
<tr>
<td>mxc²⁶⁶/Y (16)</td>
<td>Viable; HT</td>
<td>Overgrown</td>
<td>1.4 ± 0.8</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>0.7</td>
<td>72 ± 63²⁴ (24)</td>
</tr>
<tr>
<td>mxc³³⁸/Y (16)</td>
<td>Pharate lethal; HT</td>
<td>Overgrown</td>
<td>4.7 ± 1.8</td>
<td>95</td>
<td>2.7</td>
<td>2.4</td>
<td>73 ± 70²⁵ (25)</td>
</tr>
<tr>
<td>mxc³⁰⁵/Y (19)</td>
<td>Pupal lethal; SD</td>
<td>Overgrown</td>
<td>6.1 ± 2.3</td>
<td>93</td>
<td>4.3</td>
<td>2.3</td>
<td>46 ± 42²¹ (21)</td>
</tr>
<tr>
<td>mxc³³⁴/Y (24)</td>
<td>Larval/pupal lethal; SD</td>
<td>Overgrown</td>
<td>3.6 ± 2.1</td>
<td>95</td>
<td>3.3</td>
<td>1.7</td>
<td>52 ± 42² (10)</td>
</tr>
</tbody>
</table>

Two wild-type controls are included: Binsn/Y male sibling larvae of mxc/Y individuals and y¹ ac¹ z¹/Y males. mxc and mxc were induced on the y¹ ac¹ z¹ chromosome. For Binsn/Y males, data from all the experimental series were pooled. N, no. of animals used for analysis of this genotype.

Viability and phenotypes of mxc mutants. HT, adult homeotic transformations; S, male and female sterile; SD, small imaginal discs.

Hemocyte counts of larvae from outcrossed balanced mxc stocks. Results are mean blood cells/milliliter ± SE.

Significantly different from the Binsn control (P < 0.01).

Significantly different from the Binsn wild-type control (P < 0.05).

Significantly different from the y¹ ac¹ z¹ wild-type control (P < 0.05).

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**Figure 1.**—Overgrown hematopoietic organs of mxc larvae. Lymph glands from wild-type and mxc late third instar larvae carrying a Dot-LacZ enhancer trap were stained with X-Gal. Anterior lobes are to the left. (A) Wild type; (B) mxc/Y; (C) mxc/Y. Note that posterior lobes show stronger hypertrophy in C (arrowhead) than in B.

**Figure 2.**—Cell division in lymph glands and circulating hemocytes. Cell division was visualized with anti-phosphohistone H3 antibody. (A) Lymph gland chain from Binsn/Y larva. Dividing cells are detected in posterior lobes (arrow). Bar, 100 µm. (B) First and second lymph gland lobes from mxc/Y larva. Numerous dividing cells are present in both. Bar, 100 µm. (C) Mitotic figure (arrow) in circulating hemocyte from mxc/Y larva. Bar, 10 µm.
for the number of crystal cells per control larva (Table 1; LANOT et al. 2001; SORRENTINO et al. 2002). mxcG43, mxc16a-1, and mxcG46 larvae exhibited three- to fivefold increases in circulating hemocytes (Table 1). mxc blood cell types appeared similar to wild type with one possible exception: mutant larvae exhibited a significant increase (4–5% compared to <0.1% in controls) in a cell type with large pseudopod-like extensions. The cells appeared pear-shaped or spindle-shaped and were different from round plasmatocytes, even with cytoplasmic inclusions, or from flattened macrophages (Figure 3). Like phagocytic plasmatocytes, these cells absorbed particles of India ink injected into the larva (LANOT et al. 2001; Figure 3); they presented numerous cytoplasmic organelles and vacuoles and resembled mxcembd podocytes (GATEFF 1978; SHRESTHA and GATEFF 1982). Accordingly, we considered them as phagocytes, possibly with altered adhesion capacities, and called them podocytes hereafter. Mutant larvae contained 2–3% lamellocytes, whereas such cells often represented <0.1% in wild type (Table 1). Lamellocytes were already differentiated within mxc mutant lymph glands, as revealed by the lamellocyte-specific enhancer trap line l(2)113/28 (Figure 3). Staining of hemocytes from wandering larvae with anti-phospho-histone H3 antibody revealed increased mitoses in mxc mutants (Table 2; Figure 2). Among wild-type hemocytes 0.64% showed mitotic figures in good agreement with previous data (RIZKI 1978; QU et al. 1998), whereas mitoses were up to four times as frequent in mxc mutants. Hence increased proliferation is present in lymph glands and in circulating blood cells. We compared crystal cell numbers per mutant larva with two controls: the y′ ac: z′ strain in which mxcG43 and mxcG46 were induced (SANTAMARIA and RANDSHOLT 1995) and the balancer chromosome Binsn. Crystal cells, visualized by heat treatment, were fewer in all mxc contexts than in wild-type controls (Table 1; Figure 3). Similar results were observed when black cells were compared in Binsn/Y;Bc/+ and mxc/Y;Bc/+ animals (not shown). The reduction was statistically significant for mxcembd and mxc16a-1 larvae when compared to both controls (Table 1). mxcG46 and mxcG43 crystal cell numbers were statistically different only from the Binsn/Y control.

Together the data show that all four mxc mutants affect lymph gland and circulating hemocyte proliferation and differentiation. Loss-of-function alterations of mxc result in abnormal numbers of circulating larval hemocytes of both the phagocyte and the crystal cell hematopoietic lineages.

**mxc hematopoietic tissue is intrinsically overproliferating and invasive:** mxc hematopoietic phenotypes could reflect a defense response to apoptosis, which occurs in mxc imaginal discs (reviewed in DEAROLF 1998; SAGET et al. 1998). Alternatively, mxc could directly control prohemocyte and hemocyte proliferation and differentiation since l(1)mbn lymph gland cells can divide after transplantation into larvae or adults; they invade healthy tissues and can, according to GATEFF and MECHLER (1989), kill the host. To choose between these alternatives, we first compared the number of apoptotic cells in wild-type and mxc mutant lymph glands. TUNEL labeling of apoptotic cells revealed hardly any increase in cell death in lymph glands from mxc mutant animals (Figure 4). Hence mxc lymph gland overproliferation is likely not induced to compensate for cell death in the hematopoietic organs.

We performed a series of transplantation experiments, since transplantation of Drosophila cells with mutations in tumor suppressor genes into wild-type adult hosts can reveal their capacity for autonomous, uncontrolled proliferation (HANRATTY and RYERSE...
females were observed 24 hr after injection. This pro-

types of mxc are summarized in Table 3. From 50 to 100% of the transplants allowed to grow for 5 days.

Growing transplanted cells either remained localized in the abdomen of the host or invaded from part to all of the body cavity (Figure 5). Hence overproliferation of mxc lymph gland tissue is an autonomous and intrinsic characteristic of these cells. We conclude that mxc wild-

type product is directly involved in control of lymph gland and hemocyte proliferation in larvae.

mxc and Toll signaling in hematopoiesis: The Toll pathway controls insect cellular defense responses, and Toll gain of function (g.o.f.) causes hematopoietic overgrowth (Qiu et al. 1998). We compared hematopoietic phenotypes of mxc− and of Toll signal g.o.f., due to either constitutive receptor activation by TlRXA or loss of the cytoplasmic inhibitor encoded by cactus in cactS1/cactS1 hypomorphs (Table 4). Qiu et al. (1998) reported a low incidence of pseudotumors along with a 2-fold hemocyte increase in TlRXA+/+ larvae and a >10-fold one in the strong cactS1/cactS1 mutant. We found that TlRXA+/+ larvae exhibited a 3- to 5-fold increase in hemocyte numbers and that 99% presented pseudotumors; hypomorphic cactS1/cactS1 larvae showed a 3-fold hemocyte increase (Table 4). These values are in the same range as mxc-induced overproliferation. In contrast to even the most severe mxc mutants (Table 1), which exhibit only 3–5% lamellocytes, both gain-of-Toll signal contexts were associated with higher lamellocyte ratios of, respectively, 13.8 and 17.0% (similar to previous TlRXA+/+ data; Lanot et al. 2001). Spindle-shaped podocytes were also present, but in lesser amounts (4.0 and 1.1% of circulating cells, respectively).

To address how mxc related to Toll signaling in hemocyte control, we constructed mxcG43/Toll pathway double mutants. Loss of the pathway was obtained in either heteroallelic TlRXA/TlRXA larvae or tub238/tub238 animals where cytoplasmic transduction of Toll signal is blocked (Govind 1999). Results are summarized in Table 4. In agreement with Qiu et al. (1998), we found that diminished Toll signaling reduces hemocyte numbers (68 and 77% of sibling TlRXA+/+ or tub238+/+ controls, respectively). Furthermore, both Toll signal loss-of-function (l.o.f.) contexts counteracted the effects of mxcG43 l.o.f. on hemocyte production. Mean hemocyte numbers of mxcG43/Y;TlRXA/TlRXA or mxcG43/Y; tub238/tub238 lar-

### TABLE 3

<table>
<thead>
<tr>
<th>Donor cells</th>
<th>No. of hosts</th>
<th>No. of transplants (%)</th>
<th>Growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>y' mxc&lt;sub&gt;abs&lt;/sub&gt;/Y</td>
<td>111</td>
<td>79 (70)</td>
<td>49 (60)</td>
</tr>
<tr>
<td>Binsn/Y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29</td>
<td>23 (80)</td>
<td>0</td>
</tr>
<tr>
<td>y' mxcG43/Y</td>
<td>20</td>
<td>10 (50)</td>
<td>7 (70)</td>
</tr>
<tr>
<td>Binsn/Y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
<td>5 (50)</td>
<td>0</td>
</tr>
<tr>
<td>y' mxc&lt;sub&gt;abs&lt;/sub&gt;/Y</td>
<td>24</td>
<td>19 (80)</td>
<td>15 (80)</td>
</tr>
<tr>
<td>Binsn/Y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21</td>
<td>21 (100)</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Genotype of transplanted hsp83-LacZ lymph glands.
<sup>b</sup> Internal control Binsn/Y siblings for each experimental series.
<sup>c</sup> Transplanted βgal<sup>+/+</sup>/βgal<sup>−/−</sup> females.
<sup>d</sup> Transplants expressing β-galactosidase 5 days after transplantation.
<sup>e</sup> Transplants showing increased size after 5 days.

![Figure 4](image-url)
Harrison

Podocytes and lamellocytes, on the other hand, were effects of
mxc
P
(Binsn gland and hemocyte overgrowth, abnormal differentia-
tion into wild-type hosts. Lymph glands were dissected from third
instar Binsn/Y;hsps83-LacZ/+ or mxc/Y;hsps83-LacZ/+ larvae and
transplanted into the abdomen of βgalα/βgalα females. Hosts were
dissected 5 days after injection

and stained with X-Gal. (A) Transplanted wild-type cells show localized β-galactosidase expression (arrow); (B) transplanted
mxc/G43/Y cells; (C) transplanted mxc+/Y cells. The dissected abdomen in C shows that β-galactosidase-expressing cells have
partially invaded the body cavity.

vae were not statistically different from the corresponding
Binsn/Y;Tl/+ or Binsn/Y;tub238/+ internal controls, whereas mxc+/Y;Tl/+ and mxc+/Y;tub238/+ sibling lar-
vae always had more circulating hemocytes (P < 0.01).
Podocytes and lamellocytes, on the other hand, were
still more numerous in mxc+/Y;Tl/loxP/loxP and mxc+/Y;
tub238/tub238 larvae compared to Binsn/Y;Tl/+ or
Binsn/Y;tub238/+ controls. Hence for hemocyte density,
loss of Toll signal is epistatic to the loss of mxc

We conclude that loss of mxc can increase body cavity hemocyte
numbers only when the Toll pathway is functional.

mxc and JAK/STAT control of hematopoiesis: Gain
of function of the JAK kinase encoded by hopscotch can
be lethal and can induce pseudotumors, strong lymph
gland and hemocyte overgrowth, abnormal differentiation
of lamellocytes, and reduced crystal cell numbers
(Hanratty and Ryerse 1981; Hanratty and Dearolf
1993; Harrison et al. 1995; Luo et al. 1995, 1997; Lanot
et al. 2001). Some of these effects are mediated by JAK
overactivation of D-stat product and can be rescued by
loss of D-stat (Hou et al. 1996; Yan et al. 1996; Luo et
al. 1997). As for the Toll pathway, we compared the effects of mxc mutations on hematopoiesis to those of
loss or gain of function of the JAK/STAT pathway. Loss of
hop reduces cell proliferation in larval tissues such as
imaginal discs and brain (Perrimon and Mahowald
1986), but no data are, to our knowledge, available concerning hemocyte numbers and ratios in hop
amorphic animals that are larval/pupal lethals. We examined
hemocyte production in amorphic hop14/14 and hop138
males raised at 25°. Interestingly, both hop null alleles
exhibit hemocyte numbers and cell type distributions that were no different from control Binsn/Y or FM7/Y
siblings (Table 5). This result suggests that, contrary to
loss of Toll signaling, loss of hop/JAK might have no
effect on the number of circulating larval hemocytes.

TABLE 4
Hemocytes in Toll pathway and mxc mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hemocytes cells/ml (10^6)</th>
<th>Plasmatocytes (%)</th>
<th>Podocytes (%)</th>
<th>Lamellocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM3/*</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>TM3/10</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>cact12/CyO</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>cact12/</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Binsn/Y;TM6c/Tl</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Binsn/Y;Tl/loxP/loxP</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>y mx+/Y;TM6c/Tl</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>y mx+/Y;TM6c/Tl</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Binsn/Y;TM6c/tub238</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Binsn/Y;tub238/</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>y mx+/Y;tm6c/tub238</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>y mx+/Y;tm6c/tub238</td>
<td>10.1 ± 0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Effects of loss of Toll were evaluated among progeny of y mx+/Binsn;Tl/loxP/loxP/TM6c females and Binsn/
Y;TM6c/Tl/loxP/loxP/TM6c males. Effects of loss of tub were evaluated among progeny of y mx+/Binsn;tub238/TM6c females
crossed to Binsn/Y;tub238/TM6c males. *P < 0.01; significantly different from Binsn/Y control. **P < 0.01;
significantly different from internal mxc+/control category in this experiment. ***P < 0.1; cell type distribution
significantly different from Binsn/Y.

* Genotype of sibling larvae, which served as internal controls in this experimental series. N, no. of animals
used for this genotype.

* Mean hemocyte density per milliliter of hemolymph ± SE.
TABLE 5

Hemocyte phenotypes induced by loss of hop and loss of D-stat

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Cells/ml (10^6)</th>
<th>Plasmatocytes (%)</th>
<th>Podocytes (%)</th>
<th>Lamellocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM7act-GFP/Y¹</td>
<td>11</td>
<td>2.5 ± 1.0</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>y w hop¹⁰⁵/Y</td>
<td>14</td>
<td>2.2 ± 1.0</td>
<td>&gt;99</td>
<td>0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Binsn/Y²</td>
<td>10</td>
<td>2.6 ± 2.1</td>
<td>98.3</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>y hop¹³⁰⁷/Y</td>
<td>11</td>
<td>2.8 ± 2.1</td>
<td>&gt;99</td>
<td>0.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>FM7c/Y;His3-GFP/+ a</td>
<td>17</td>
<td>1.1 ± 0.4</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>FM7c/Y;D-stat6346/+</td>
<td>17</td>
<td>1.0 ± 0.6</td>
<td>&gt;99</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>hop¹sw/Y;His3-GFP/+</td>
<td>22</td>
<td>15.1 ± 7.6</td>
<td>63.2</td>
<td>11.4</td>
<td>25.4</td>
</tr>
<tr>
<td>hop¹sw/Y;D-stat6346/+</td>
<td>23</td>
<td>6.2 ± 3.6*</td>
<td>77.1**</td>
<td>5.6**</td>
<td>17.3**</td>
</tr>
</tbody>
</table>

hop¹sw/FM7c females were mated to +/Y;D-stat6346:/His3-GFP males. Data from two independent experiments were pooled. *P < 0.01; significantly different from hop¹sw/Y;His3-GFP/+; **P < 0.01; cell type distribution significantly different from hop¹sw/Y;His3-GFP/+.

a Genotype of sibling larvae, which served as internal wild-type controls in this experiment. N, no. of animals used for this genotype.

¹ Mean hemocyte density per milliliter, ± SE.

Hou et al. (1996), Yan et al. (1996), and Luo et al. (1997) have reported partial suppression of g.o.f. hop-associated lethality and pseudotumors by mutations of D-stat, mainly at 29°. Furthermore, Luo et al. (1997) reported inhibition of hop¹sw/Y-associated lamellocyte differentiation by loss of a D-stat copy. To establish whether similar effects are observed at 25°, we examined plasmatocyte and lamellocyte production in a hop¹sw/Y;D-stat6346/+ context at this temperature. In agreement with the authors cited above, we found that hop¹sw/Y induced strong hemocyte overproduction and that 25% of these were lamellocytes; pseudotumors were less frequent in female hop¹sw/+;D-stat6346/+ larvae compared to their hop¹sw/+/His3-GFP/+ siblings at this semirestrictive temperature (10 and 24%, respectively). Interestingly, larvae raised at 25° revealed a suppressive effect of loss of D-stat on hop¹sw/Y hemocyte numbers since hop¹sw/Y;D-stat6346/+ males had significantly fewer circulating blood cells than did their hop¹sw/Y;His3-GFP/+ siblings (Table 5). Hence D-stat product could be required for both proliferation and lamellocyte differentiation in response to hop¹sw/encoded product. D-stat6346 null mutants are larval/pupal lethals and exhibit small imaginal discs, indicating that D-stat plays a role in control of imaginal cell proliferation (Hou et al. 1996). Our data suggest that this role extends to hemocyte proliferation.

We first looked for mxe and JAK/STAT pathway interactions in mxe⁶⁴⁷/Y;D-stat6346/+ larvae. Total circulating hemocyte numbers as well as lamellocyte numbers were similar in mxe⁶⁴⁷/Y;TM6c/+ and mxe⁶⁴⁷/Y;D-stat6346/+ larvae (Table 6). Similar results were obtained for mxe⁶⁴⁷/Y;D-stat⁶⁰⁸/D-stat6346 larvae compared to mxe⁶⁴⁷/Y;D-stat⁶⁰⁸/+ animals (data not shown). Hence mxe effects on blood cell numbers are likely not modified by loss of D-stat.

We wondered whether the same held true for Tl10b and assessed this by looking for effects of D-stat6346 in trans with Tl10b. Heterozygosity for D-stat6346 reduced lamellocyte differentiation in Tl10b/D-stat6346 compared to Tl10b/His3-GFP larvae (Table 6). Therefore the strong induction of lamellocyte differentiation caused by gain of Toll requires D-stat.

To conclude, our data indicate that hemocyte production and lamellocyte differentiation in various genetic and experimental contexts show different sensitivities to changes in D-stat dosage. D-stat quantity is apparently limiting for the strong hemocyte production induced by constitutive hop/JAK activation at 25°, whereas the lesser blood cell productions of mxe⁶⁴⁷ or Tl10b are not affected by D-stat hemizygosity. Furthermore, as previously reported for hop¹sw/lamellocyte production (Luo et al. 1997), we find that the strong lamellocyte differentiation in Tl10b also depends on D-stat product, whereas the lesser lamellocyte production of mxe⁶⁴⁷ is unaffected in both a D-stat6346/+ and a D-stat⁶⁴⁷/D-stat6346 context.

Lymph gland proliferation and circulating hemocytes in double inductive conditions: We examined the effects on hemocyte production of several double mutant contexts that each alone increase hemocyte numbers. Interestingly, mxe⁶⁴⁷/Y;Tl10b/+ and mxe⁶⁴⁷/Y;cact⁶⁴²/cact⁶⁴² larvae had fewer hemocytes than did Tl10b/+ , mxe⁶⁴⁷/Y, or cact⁶⁴²/cact⁶⁴² animals (Table 7; P < 0.01). Furthermore, joint activation of the Toll and the JAK/STAT pathways had comparable effects in hop¹sw/Y;Tl10b/+ and hop¹sw/Y;cact⁶⁴²/cact⁶⁴² larvae (not shown). mxe⁶⁴⁷ hop¹sw/Y larvae had 3.1 × 10⁶ (SD ± 1.3 × 10⁶) hemocytes/ml, which represents an increase compared to the internal wild-type control (0.7 ± 0.4 × 10⁶ hemocytes/ml), but is considerably less than the added effects of mxe⁶⁴⁷ and hop¹sw alone. All such double mutant animals contained...
abnormally large hemocytes. To understand this phenomenon, we examined lymph glands from mxcG43 hopTum-l Y and mxcG43/Tl10b cacta2/cacta2 animals. Such hematopoietic organs were very fragile and difficult to dissect. When the lymph glands could be isolated, they showed severe overgrowth and contained high numbers of differentiated blood cells (mxcG43/Y;cacta2/cacta2 in Figure 6). This was also associated with intense mitotic activity, as revealed by staining with anti-phosphohistone H3 antibody (Figure 6). TUNEL label revealed no increase in apoptotic cell death in these lymph glands (mxcG43/Y; cacta2/cacta2 example in Figure 6). Lamellocytes differentiated within the hematopoietic organs; in some larvae, the glands were covered by lamellocytes, as shown by the lamellocyte-specific reporter b(2)113/28, and progressively melanized (Figure 6). Together these data suggest that differentiated blood cells in the dramatically overgrown lymph gland lobes are not released into the hemolymph. The lymph glands could end by being recognized as non-self and encapsulated by the cellular host defense system of the animal.

mxc, PcG, and trxG genes and larval hemocyte control: During Drosophila and vertebrate development, trxG and PcG genes control the expression of many targets, including the Hox genes. Furthermore, mammalian hematopoiesis is a target of trxG and PcG regulation (reviewed in Takihara and Harha 2000; Muller and Leutz 2001; Raaphorst et al. 2001). Hence, we asked whether Drosophila larval hematopoiesis depended on PcG and trxG gene products to the same extent as ho-

### TABLE 6

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Cells/ml (10⁶)</th>
<th>Plasmocytes (%)</th>
<th>Podocytes (%)</th>
<th>Lamellocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binsn/Y;His3-GFP/+</td>
<td>10</td>
<td>0.6 ± 0.2</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Binsn/Y:D-stat6346/+</td>
<td>8</td>
<td>0.5 ± 0.3</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>y¹ mxcG43/Y;His3-GFP/+</td>
<td>11</td>
<td>1.4 ± 0.6*</td>
<td>89.5</td>
<td>8.5</td>
<td>2.0</td>
</tr>
<tr>
<td>y¹ mxcG43/Y;D-stat6346/+</td>
<td>11</td>
<td>1.8 ± 0.9*</td>
<td>92.5</td>
<td>6.5</td>
<td>1.0</td>
</tr>
<tr>
<td>+/Y;TM6c/His3-GFP</td>
<td>14</td>
<td>0.4 ± 0.3</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>+/Y;TM6c/TM6c</td>
<td>16</td>
<td>2.2 ± 0.9*</td>
<td>84.0</td>
<td>0.1</td>
<td>15.8</td>
</tr>
<tr>
<td>+/Y;D-stat6346/His3-GFP</td>
<td>15</td>
<td>0.3 ± 0.2</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>+/Y;TM6c/D-stat6346</td>
<td>16</td>
<td>3.5 ± 1.0*</td>
<td>95.6**</td>
<td>1.4**</td>
<td>3.3**</td>
</tr>
</tbody>
</table>

Effects of D-stat6346 on mxcG43 were examined in male progeny from +/Y;D-stat6346/His3-GFP males mated with y¹ mxcG43/Binsn females. D-stat6346 and TM6c interactions were examined among male progeny of +/Y;TM6c/His3-GFP males and +/+;D-stat6346/TM6c females. N, no. of animals utilized for this genotype. *P < 0.01; significantly different from internal control. **P < 0.01; blood cell type distribution significantly different from +/Y;TM6c/TM6c.

a Genotype of sibling larvae, which served as internal wild-type control in this experiment.

b Mean hemocyte no. per milliliter ± SE.

### TABLE 7

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Cells/ml (10⁶)</th>
<th>Plasmocytes (%)</th>
<th>Podocytes (%)</th>
<th>Lamellocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binsn/Y;cacta2/CyO act-GFP</td>
<td>10</td>
<td>1.7 ± 0.7</td>
<td>&gt;99</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>y¹ mxcG43/Y;cacta2/CyO act-GFP</td>
<td>10</td>
<td>5.0 ± 2.0*</td>
<td>92.9</td>
<td>5.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Binsn/Y;cacta2/cacta2</td>
<td>10</td>
<td>5.1 ± 1.5*</td>
<td>88.8</td>
<td>1.3</td>
<td>9.9</td>
</tr>
<tr>
<td>y¹ mxcG43/Y;cacta2/cacta2</td>
<td>10</td>
<td>1.9 ± 0.9</td>
<td>86.3</td>
<td>8.5</td>
<td>6.2</td>
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<tr>
<td>Binsn/Y;His3-GFP/+</td>
<td>10</td>
<td>0.7 ± 0.3</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
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<tr>
<td>y¹ mxcG43/Y;His3-GFP/+</td>
<td>15</td>
<td>3.0 ± 1.4*</td>
<td>88.8</td>
<td>8.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Binsn/Y;TM6c/His3-GFP/+</td>
<td>9</td>
<td>3.9 ± 1.3*</td>
<td>93.0</td>
<td>1.0</td>
<td>6.0</td>
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<tr>
<td>y¹ mxcG43/Y;TM6c/His3-GFP/+</td>
<td>13</td>
<td>1.8 ± 1.0</td>
<td>73.8</td>
<td>23.8</td>
<td>2.4</td>
</tr>
</tbody>
</table>

y¹ mxcG43/Y;cacta2 animals were progeny of y¹ mxcG43/Binsn;cacta2/CyO act-GFP females and Binsn/Y;cacta2/CyO act-GFP males. mxcG43/Y;TM6c/His3-GFP/+ larvae were progeny of y¹ mxcG43/Binsn females and +/Y;TM6c/His3-GFP males. N, no. of larvae utilized for this genotype. *P < 0.01; significantly different from internal control.

a Genotype of sibling larvae, which served as internal wild-type controls in this experimental series.

b Mean hemocyte no. per milliliter ± SE.
meiotic genes do for identity specification along the anterior/posterior body axis. Indeed, homeotic transformations of single PcG mutants are synergistically enhanced by adding a second PcG mutation in trans (Jürgens 1985), whereas trxG mutations in trans suppress PcG mutant phenotypes (Kennison and Tamkun 1988). Homoeocytes were counted for mxc^{89}/Y; esc^{2}/esc^{2} larvae, with anti-phosphohistone H3 antibody. (B) Enlarged view of detail of lymph gland in A reveals anti-phosphohistone H3 antibody label in dividing cells. (C) Detail of TUNEL-labeled mxc^{89} hop^{Xen}/Y lymph gland. Apoptotic cells are indicated by arrowheads. (D) mxc^{89} hop^{Xen} lymph glands contain differentiated hemocytes: lymph glands were squashed with a coverslip after dissection in 1× PBS. (E) Lamellocytes differentiate inside overgrown mxc^{89} hop^{Xen}/Y lymph glands, revealed by β-galactosidase expression of the l(2)113/28 reporter line. (F) X-Gal-stained mxc^{89} hop^{Xen}/Y; l(2)113/28 / + lymph gland lobe, covered by lamellocytes and partially melanized (arrowhead); same scale as E. Bars in A and E, 150 μm; bars in B, C, and D, 40 μm.

FIGURE 6.—Lymph glands in mxc;act and mxc;hop^{Xen} double mutants. (A) Whole view of dramatically overgrown lymph gland chain from mxc^{89}/Y;act^{2}/act^{2} larva, stained with anti-phosphohistone H3 antibody. (B) Enlarged view of detail of lymph gland in A reveals anti-phosphohistone H3 antibody label in dividing cells. (C) Detail of TUNEL-labeled mxc^{89} hop^{Xen}/Y lymph gland. Apoptotic cells are indicated by arrowheads. (D) mxc^{89} hop^{Xen} lymph glands contain differentiated hemocytes; lymph glands were squashed with a coverslip after dissection in 1× PBS. (E) Lamellocytes differentiate inside overgrown mxc^{89} hop^{Xen}/Y lymph glands, revealed by β-galactosidase expression of the l(2)113/28 reporter line. (F) X-Gal-stained mxc^{89} hop^{Xen}/Y; l(2)113/28 / + lymph gland lobe, covered by lamellocytes and partially melanized (arrowhead); same scale as E. Bars in A and E, 150 μm; bars in B, C, and D, 40 μm.

meiotic genes do for identity specification along the anterior/posterior body axis. Indeed, homeotic transformations of single PcG mutants are synergistically enhanced by adding a second PcG mutation in trans (Jürgens 1985), whereas trxG mutations in trans suppress PcG mutant phenotypes (Kennison and Tamkun 1988). Homoeocytes were counted for mxc^{89}/Y in trans with alleles of Sex comb on Midleg (Sem), Polycomb-like (Pcl), Polycomb (Pc), and Posterior sex combs (Psc), which strongly enhance adult homeotic transformations of the viable mxc^{89} allele (Saget et al. 1998). We also tested loss of extra sex combs (esc). None of the five mutations affected control or mxc^{89} homoeocyte numbers or ratios (data not shown). This suggests that Drosophila PcG genes are required differently in larval homeocyte production and in HOM/Hox gene regulation. Indeed, mxc, Pcl, Pc, Sem, and Psc exert common negative control on the latter process, whereas mxc alone seems critically required for the former.

Implication of trxG products in hematopoiesis was
tested in trans-heterozygous mxc;trxG genetic contexts, using trithorax (trx), moira (mor), and brahma (brm) mutations that counteract PcG homeotic phenotypes in trans (FlyBase 1999). trx and mor mutations had no effect on wild-type or mxc homoeocyte numbers and ratios. Interestingly, whereas lymph glands of brm^{2}/+ were no different from wild type (not shown), loss of brm reduced circulating hemocytes in brm^{2}/+ and in y^{+} mxc^{89}/Y;brm^{2}/+ larvae (Table 8). Similar results were observed for brm^{2} trx^{2}/+ and y^{+} mxc^{89}/Y;brm^{2} trx^{2}/+ larvae, indicating that brm alone affects circulating hemocyte numbers. The trxG protein BRM is similar to yeast SWI/SNF chromatin-remodeling proteins. The Drosophila gene domino also encodes SWI/SNF chromatin proteins that are, like their mammalian homologs, involved in control of cell proliferation (reviewed in Gebauer et al. 2000; Ruhf et al. 2001). To further characterize the role of brm in hematopoiesis, we examined hematocyte numbers in Tl^{p106}+/+ and hop^{Xen}/Y larvae carrying a brm^{2} allele (Table 8). The brm^{2}+/+ context had no effect on Tl^{p106}+/+ phenotype, whereas hop^{Xen}/Y; brm^{2}+/+ larvae contained significantly fewer hemocytes than did their hop^{Xen}/Y; His3-GFP+/+ siblings (P < 0.01). Lamellocyte ratios remained unchanged (respectively, 28.3% and 28.4%); hence brm^{2} does not block hop^{Xen}-induced lamellocyte differentiation. Together, these data show, first, that two Drosophila SWI/SNF proteins, BRM and DOM, participate in hematopoiesis control and, second, that upregulation of hematopoietic production by Toll is less sensitive to the level of brm product than upregulation either by activation of JAK or by loss of mxc.

DISCUSSION

**mxc controls blood cell proliferation and differentiation**:

Here, we confirm that wild-type mxc directly regulates hematocyte proliferation and differentiation in Drosophila larvae. Gateff and Mechner (1989) reported modification of these processes by mxc l(l)mbl alleles, which were screened as causing hyperplasia and neoplasia. We examined hematopoietic phenotypes of three mutants that were isolated on the basis of other criteria (Santamaria and Randsholt 1995; Docquier et al. 1996). Hematopoiesis was not analyzed in strong mxc mutants, because hyperplasia and neoplasia were not seen in circulating cells. Lymph gland overgrowth is progressively stronger from mxc^{89} to mxc^{89}/Y (Table 1) and affects more and more posterior lobes, in good agreement with Shrestha and Gateff (1982) who described the whole hematopoietic organ of mxc^{89}/Y as hy-
perplastic. Lanot et al. (2001) proposed that posterior lymph gland lobes contain hematopoietic blast cells, similar to bone marrow cells, which can be solicited to differentiate into given blood cell lines. Our data support the notion that anterior lobe prohemocytes are more readily solicited to divide and differentiate than are posterior ones.

Interestingly, the stronger mxc alleles showed lower numbers of circulating crystal cells that are about 10 times less frequent in mxc<sup>abd</sup> and mxc<sup>mot</sup> larvae compared to wild type. It has been suggested that crystal cell numbers might also be reduced in hop<sup>trans</sup>/Y animals at non-permissive temperatures (Lanot et al. 2001). This could in both cases reflect recruitment of crystal cells in the melanotic capsules of mutant larvae. Still, such capsules were far from always detected in mxc larvae, since only up to 25% mxc<sup>abd</sup>/Y animals developed pseudotumors. Hence mxc might affect crystal cell development. During hematopoiesis, plasmatocytes and crystal cells develop from a common pool of cells expressing the GATA protein Serpent (reviewed in Fossett and Schulz 2001; Meister and Govind 2002). The plasmatocyte cell lineage is specified by the glial cells missing (GCM) conserved transcription factor; crystal cell lineage development depends on expression of the Acute Myeloid Leukemia 1 (AML-1)-like transcription factor Lozenge and is repressed by the friend of GATA protein U-shaped (USH; Lebestky et al. 2000; Fossett et al. 2001; Fossett and Schulz 2001). As some crystal cells are still present in the most severe mxc mutant, we propose that procrys-tal cell proliferation or differentiation into crystal cells, rather than crystal cell lineage specification per se, might be affected. Future investigation will determine whether modifications of mxc product levels also affect crystal cell precursors in the lymph glands and, if so, through which targets.

**Phagocytic cell types in mxc mutants:** Circulating hemocytes in mxc larvae consistently exhibited several percent of phagocytic cells, which we called podocytes (Figure 2), like the phagocytic cells described by Shrestha and Gateff (1982) as a prevailing cell type in mxc<sup>abd</sup> larvae. Like lamellocytes, such podocytes are rarely seen in wild-type larvae (<0.1% of circulating cells). We examined wild-type hemocytes from mid-third instar until 20 hr after pupariation without finding large amounts of similar cells. Changes in phagocytic cells from rounded plasmatocytes to flattened macrophages with membranous extensions and changed adhesion capacities normally occur at the end of the third instar and can be induced before by increasing ecysone titer (Lanot et al. 2001). As suggested by Rizki (1978), podocytes might represent an intermediary form between these phagocytic cell types, which would differentiate in greater numbers in mxc mutants before pupariation. Their spindle shape suggests changed adhesion features and raises the question whether these cells could play an invasive role in mxc mutants.

**mxc invasiveness and neoplasia:** mxc hematopoietic tissue from different alleles divides autonomously when transplanted into a wild-type host. Proliferative capaci-

### Table 8

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Cells/ml (10&lt;sup&gt;6&lt;/sup&gt;)</th>
<th>Plasmatocytes (%)</th>
<th>Podocytes (%)</th>
<th>Lamellocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BinSn/Y;His3-GFP/+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
<td>0.8 ± 0.4</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>BinSn/Y;brm&lt;sup&gt;mot&lt;/sup&gt;/+</td>
<td>24</td>
<td>0.4 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>y&lt;sup&gt;i&lt;/sup&gt;mxc&lt;sup&gt;mot&lt;/sup&gt;/Y;His3-GFP/+</td>
<td>18</td>
<td>3.4 ± 0.8</td>
<td>93.5</td>
<td>5.0</td>
<td>1.5</td>
</tr>
<tr>
<td>y&lt;sup&gt;i&lt;/sup&gt;mxc&lt;sup&gt;mot&lt;/sup&gt;/Y;brm&lt;sup&gt;mot&lt;/sup&gt;/+</td>
<td>25</td>
<td>1.4 ± 0.7&lt;sup&gt;****&lt;/sup&gt;</td>
<td>93.3</td>
<td>3.9</td>
<td>2.8</td>
</tr>
<tr>
<td>+/Y;His3-GFP/TM6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>11</td>
<td>0.7 ± 0.5</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>+/Y;brm&lt;sup&gt;mot&lt;/sup&gt;/His3-GFP</td>
<td>13</td>
<td>0.4 ± 0.3</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>+/Y;TF&lt;sup&gt;trans&lt;/sup&gt;/TM6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12</td>
<td>1.4 ± 0.8&lt;sup&gt;*&lt;/sup&gt;</td>
<td>81.4</td>
<td>7.0</td>
<td>12.6</td>
</tr>
<tr>
<td>+/Y;brm&lt;sup&gt;mot&lt;/sup&gt;/TF&lt;sup&gt;trans&lt;/sup&gt;</td>
<td>14</td>
<td>1.4 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.8</td>
<td>7.8</td>
<td>14.6</td>
</tr>
<tr>
<td>FM7c/Y;+/TM6&lt;sup*e&lt;/sup&gt;</td>
<td>16</td>
<td>1.0 ± 0.3</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>FM7c/Y;brm&lt;sup&gt;mot&lt;/sup&gt;/+</td>
<td>19</td>
<td>0.7 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;99</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>hop&lt;sup&gt;trans&lt;/sup&gt;/Y;+&lt;sup&gt;/&lt;/sup&gt;/TM6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>15</td>
<td>5.7 ± 4.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>42.2</td>
<td>29.4</td>
<td>28.4</td>
</tr>
<tr>
<td>hop&lt;sup&gt;trans&lt;/sup&gt;/Y;brm&lt;sup&gt;mot&lt;/sup&gt;/+</td>
<td>17</td>
<td>2.7 ± 1.7&lt;sup&gt;****&lt;/sup&gt;</td>
<td>48.0</td>
<td>23.7</td>
<td>28.3</td>
</tr>
</tbody>
</table>

For interactions with brm<sup>mot</sup>/Y;BinSn females were crossed to +/Y;brm<sup>mot</sup>/His3-GFP males; results from two experiments were pooled. brm<sup>mot</sup>/TM6<sup>e</sup> females were crossed to TF<sup>trans</sup>/His3-GFP males. hop<sup>trans</sup>/FM7c females were mated with brm<sup>mot</sup>/TM6<sup>e</sup> males and data from two experiments were pooled. *P < 0.05; significantly different from wild-type control. **P < 0.01; significantly different from wild-type control. ***P < 0.01; significantly different from mxc<sup>mot</sup>/Y;His3-GFP/+ . ****P < 0.05; significantly different from hop<sup>trans</sup>/Y;+<sup>/</sup>/TM6<sup>e</sup>.

* Genotype of sibling larvae, which served as internal wild-type controls in this experiment. N, no. of animals analyzed for this genotype.

* Mean no. of hemocytes per milliliter ± SE.
ties of mxc lymph glands appear higher than those of larval brain or imaginal disc cells mutated for tumor suppressor genes. Indeed, growth of lethal giant larvae, brain tumor, or discs large transplants required 9–12 days at 25°C (Woodhouse et al. 1998), whereas mxc transplants increased after 5 days at 23°C. hop<sup>h1034</sup> lymph gland cells, grown at the restrictive temperature of 29°C before and after transplantation, also increase after 3–5 days in wild-type females (Hanratty and Ryrse 1981). This might reflect differences in proliferation control mechanisms between the loosely structured lymph gland tissue where cell division is inducible and the highly structured larval disc and brain tissue (Harrison et al. 1995). Such differences in growth control might explain why mxc and other hematopoiesis mutants exhibit blood cell overproliferation together with reduced imaginal discs and brains (Watson et al. 1991; Torok et al. 1993; Saget et al. 1998).

Contrary to the data reported by Gattef and Mechler (1989), we found no lessened viability in females transplanted with mxc lymph glands, even 3 weeks after transplantation. This result was unexpected for the mxc<sup>h1034</sup> and mxc<sup>h1031</sup> pupal lethal alleles and less so for mxc<sup>G46</sup>, which shows partial viability at 23°C. As mxc mutations are temperature sensitive (Saget et al. 1998), 23°C may not induce sufficient proliferation to endanger the transplanted hosts. Indeed, at 29°C hop<sup>h1031</sup> is lethal and transplanted hop<sup>h1031</sup> lymph gland tissue kills a wild-type host, whereas hop<sup>h1031</sup> animals are viable at 25°C. We propose that loss of mxc function leads to hematopoietic neoplasia, invasiveness, and altered blood cell composition but not necessarily to a lethal malignant transformation.

Hematopoietic defects and other mxc phenotypes: Severity of hematopoietic defects was correlated with that of other mxc phenotypes. Previous genetic studies showed mxc<sup>h106</sup> as a viable and mxc<sup>G43</sup> as a medium severe allele, whereas mxc<sup>h106</sup> is a very strong and mxc<sup>h101</sup> an even stronger hypomorph. mxc adult males exhibit ANT-C and BX-C gene gain-of-function-like homeotic transformations, with increasing penetrance and expressivity from mxc<sup>h11</sup> via mxc<sup>h106</sup> to mxc<sup>G43</sup> (Santamaria and Randsholt 1995). mxc<sup>h1050</sup>, mxc<sup>h101</sup>, and mxc<sup>h101</sup> larvae die before the pharate stage with ectopic homeotic gene expression in many discs (Saget et al. 1998), hence strong HOM gene deregulation is also present in these mutants. Severity of germline proliferation defects increases similarly from partial mxc<sup>h11</sup> sterility to reduced mxc<sup>G43</sup> gonads in males and to almost no detectable gonad development in mxc<sup>h101</sup> larvae (Docquier et al. 1996). mxc animals show increasing developmental delays from mxc<sup>h11</sup> to mxc<sup>h101</sup> (the strongest allele that reaches the third larval instar), whereas imaginal discs and brains of some mxc<sup>h101</sup> and all mxc<sup>h101</sup> larvae are reduced in size and amorphic mxc alleles are lethal in clones (Saget et al. 1998). Mitotic figures or metaphase chromosome morphology are not visibly affected by loss of mxc (Saget et al. 1998), hence mxc<sup>+</sup> could have a more subtle effect on cell division rates, possibly through the cell cycle. Interestingly, recent data suggest several links between PcG function and regulation of the cell cycle (reviewed in Brock and van Lohuizen 2001).

For pseudotumour formation, lymph gland overgrowth, and mitosis rate in circulating hemocytes, we found increasing severity from mxc<sup>G46</sup>, mxc<sup>G43</sup>, mxc<sup>h101</sup> to mxc<sup>h101</sup>. The exception was the lower number of circulating hemocytes in mxc<sup>h101</sup> compared to mxc<sup>h101</sup> larvae (Table 1). Proliferation and survival of larval imaginal cells are more affected by mxc<sup>h101</sup> than by mxc<sup>h101</sup> (Saget et al. 1998), hence this lower hemocyte density might reflect the final outcome of increased lymph gland and hemolymph cell division together with lesser survival of circulating hemocytes. Alternatively, mxc<sup>h101</sup> might affect hemocyte identity in the lymph glands more than mxc<sup>h101</sup>, and fewer mxc<sup>h101</sup> cells would consequently be released into the body cavity (see below).

**mxc function in relation to the Toll pathway:** Several mutant phenotypes suggested that mxc and the Toll pathway could regulate a common set of processes. First, both gain of Toll signaling and mxc mutations induce lymph gland and hemocyte overproliferation. Furthermore, mxc and cact mutants exhibit decreased adhesion among fat body cells, while overexpression of the target of Toll signal Dorsal/Rel and loss of mxc both induce salivary gland atrophy (Qiu et al. 1998; O. Saget, personal communication). Our genetic analysis shows that loss of Toll signal is epistatic over loss of mxc in circulating hemocyte production, indicating that increased hemocyte numbers in mxc mutants require the Toll pathway. But Toll signal g.o.f. and mxc phenotypes also show striking differences since lamellocyte differentiation is more strongly induced and pseudotumors are much more frequent in Toll g.o.f. larvae. A further difference is that crystal cell numbers are not affected in Toll g.o.f. mutants (Lanot et al. 2001). We interpret these differences as an indication that Toll signal and mxc represent different inputs, which both regulate wild-type hemocyte production. Loss of the Toll input could render lymph gland cells unable to respond to loss of mxc.

**hop and D-stat in hematopoiesis control:** Contrary to a study of larvae with lessened Toll signal (Qiu et al. 1998), we found no modification of circulating hemocyte numbers in wandering larvae with total loss of hop. Amorphic hop larvae survive until the late larval/pupal stage because of maternal hop product perdurance (Perrimon and Mahowald 1986). Hence wandering hop<sup>−</sup> larvae may not be sufficiently depleted of hop/JAK to show defective hemocyte production. Alternatively, this result could support the view (Luo et al. 1997) that in plasmatocytes hop/JAK regulates larval capacity to respond to proliferative and differentiative signals. Therefore, only the mutant, overactive forms of hop/JAK encoded by hop<sup>h101</sup> and hop<sup>G43</sup> (Luo et al. 1997) would affect hemocyte production. Loss of hop might not change
control of hematopoiesis but could render the system less able to respond to an infection or immune challenge.

Our comparison between hop<sup>dom1</sup>/Y and hop<sup>dom1</sup>/Y; D-stat<sup>6346</sup>/+ males (as well as hop<sup>dom1</sup>/+ and hop<sup>dom1</sup>/+; D-stat<sup>6346</sup>/+ females) revealed that D-stat product is involved in hop g.o.f. plasmatocyte overproliferation. This possibility was previously suggested by Zeidler et al. (2000) and is upheld by the fact that D-STAT can bind to the D-raf promoter and activate its transcription (Kwon et al. 2000). Luo et al. (2002) recently reported that JAK g.o.f.-mediated hemocyte overproliferation and lamellocyte differentiation both require the D-Raf/D-MEK/mitogen-activated protein kinase pathway, linking again in a common regulatory network these two tense imaginal cell death (1996; et al. D-Raf that JAK g.o.f.-mediated hemocyte overproliferation hemocytes could be attached to the imaginal discs and hop D-stat partially suppresses pseudotumors and lamellocyte proliferation and differentiation of hematopoietic cells (Nosaka et al. 1999; reviewed in Luo and Dearolf 2001). We observed a critical requirement for D-stat product on hemocyte numbers only in the hop g.o.f. context at 25°; indeed, loss of D-stat in D-stat<sup>dom1</sup>/D-stat<sup>6346</sup> larvae had no effect on hemocyte numbers of mxc<sup>dom1</sup>/Y, and loss of one D-stat copy had no effect on plasmatocyte numbers of T<sup>dom1</sup>/Y larvae. Gain of hop induced a >10-fold increase in circulating hemocytes whereas the gain-of-Toll or loss-of-mxc contexts that we examined induced but 3- to 4-fold increases. Therefore, this apparent difference in sensitivity to D-stat dosage could reflect a threshold situation, where only stronger hemocyte overproductions are visibly affected when D-stat is reduced.

Lamellocyte differentiation and D-stat: Mutation of D-stat partially suppresses pseudotumors and lamellocyte differentiation in hop g.o.f. (Hou et al. 1996; Yan et al. 1996; Luo et al. 1997; this article). Lamellocyte differentiation induced in T<sup>dom1</sup>/+ larvae also depends on D-stat dosage (Table 5). We were unable to establish whether T<sup>dom1</sup>-induced lamellocyte differentiation depends on hop/JAK, since hop<sup>dom1</sup>/Y; T<sup>dom1</sup>/+ larvae, in which JAK is absent, died before the third instar. As heterozygosity for D-stat suppresses +/T<sup>dom1</sup> lamellocyte production, we propose that the Toll pathway is upstream of JAK/STAT signaling in this process. This would confirm the hypothesis of Mathey-Prevot and Perrimon (1998) who speculated that Toll might be upstream of JAK/STAT in hemocyte differentiation. Lagèuex et al. (2000) have recently shown similar sequential effects of these two pathways on induction of a complement-like protein, which could have important roles in defense response to infection of Drosophila larvae. As argued above, the lack of effect of D-stat mutation in mxc<sup>dom1</sup> larvae could indicate that lamellocyte production in this mutant was too low to be sensitive to a reduction in D-stat product.

Lymph glands and circulating hemocytes in double stimulated hematopoiesis conditions: Double-mutant contexts, which each alone increase hemocyte production, yielded intriguing results of reduced hemocyte numbers compared to single mutants. This was true for combinations associating mxc with gain of function of Toll or of JAK, but also for double Toll signal and JAK g.o.f. contexts. The double-mutant animals contained abnormally large hemocytes with numerous inclusions or vacuoles and showed delayed development (24–48 hr in wandering larvae). This could reflect a lesser circulating hemocyte production, as in severe domino mutants (Braun et al. 1997, 1998; Ruhf et al. 2001). Alternatively, hemocytes could be attached to the imaginal discs and involved in phagocytosis, as in proliferation disruptor (prod) mutants that show delayed development and intense imaginal cell death (Torok et al. 1997). Lymph glands from mxc<sup>dom1</sup> hop<sup>dom1</sup>/Y and mxc<sup>dom1</sup>/Y; cact<sup>dom1</sup>/cact<sup>dom1</sup> larvae showed dramatic overgrowth and extreme fragility, associated with intense mitotic activity and the presence of numerous differentiated hemocytes in the glands. The enlarged lymph glands were sometimes encapsulated by lamellocytes and melanized, recalling phenotypes of medium severe domino mutants that show massive overgrowth and blackening of the lymph glands together with rare, abnormally large circulating hemocytes (Ruhf et al. 2001). Recently, Luo et al. (2002) reported that hop g.o.f. animals without a functional D-raf pathway also exhibit overgrown lymph glands and dramatic reductions in circulating blood cell numbers and attributed this phenotype to a requirement of D-raf signaling for cell survival in the lymph glands, downstream of hop g.o.f.-induced proliferation. All these phenotypes illustrate that the final number of circulating larval hemocytes depends on several processes in the lymph glands, including control of prohemocyte division rates and of hemocyte differentiation (hop, D-stat, Toll, and mxc) but also hemocyte survival (dom, D-raf, Braun et al. 1997, 1998; Luo et al. 2002) and hemocyte capacity to cross the basement membrane (dom). A common explanation for all these data could be that strong deregulation of one or joint deregulation of any two of these processes could lead to production of cells whose modified characteristics (cell surfaces) hinder their passage into the body cavity or whose modified identities prevent survival. Overgrown mxc<sup>dom1</sup> hop<sup>dom1</sup>/Y or mxc<sup>dom1</sup>/Y; cact<sup>dom1</sup>/cact<sup>dom1</sup> lymph glands showed numerous differentiated hemocytes and no enhanced cell death, suggesting that basement membrane passage rather than cell survival is affected in these animals. On the other hand, hemocyte identities would be so modified in strong dom<sup>1</sup> mutant larvae that cell survival is impossible, hence the lack of circulating hemocytes and melanized lymph glands containing abnormal dying cells in these animals (Braun et al. 1997). This agrees with the fact that dom<sup>1</sup> is not rescued by stimulated hemocyte production, as in dom<sup>1</sup>/dom<sup>2</sup>; T<sup>dom1</sup> + , hop<sup>dom1</sup>/Y; dom<sup>1</sup>/dom<sup>1</sup> and dom<sup>1</sup>/dom<sup>2</sup>; cact<sup>dom1</sup>/cact<sup>dom1</sup> animals (Braun et al. 1998) or in
production.

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trithorax Lohuizen 1999) and MLL
Ruhf a number of these steps, which have no Drosophila (
and cell types (reviewed in
different cell lineages and differentiation of many tissues
malian hematopoiesis involves specification of many dif-
proliferation and maintains lymph gland cell identities

dent of other PcG gene products (mxc
defects behave like mxc germline proliferation defects,
which according to genetic analysis are also independent
of other PcG gene products (Docquier et al. 1996).
Some of these PcG genes may play a role in larval hematopoi-
esis, since we examined only trans-heterozygous mu-
tant contexts. But in contrast to their role in segmental
identity specification, no haplo-insufficiency for such an
effect was detected in hematopoiesis. Hence Drosophila
requirements for PcG gene products are different in
A/P identity specification and in hematopoiesis. As mam-
malian hematopoiesis involves specification of many dif-
cent cell lineages and differentiation of many tissues
and cell types (reviewed in Orkin 2000), PcG genes may
well have been recruited during evolution to control
a number of these steps, which have no Drosophila
equivalents. In agreement with this, a recent review
(Raaphorst et al. 2001) underlined the role of PcG
genes as regulators of mammalian lymphopoiesis.

Three trxG genes were tested for effects on hemocyte
production. trx mutations had no effect on hemocyte
numbers either, although overexpression of the human
trithorax homolog MLL is associated with many acute
myeloid or lymphoblastic leukemias (reviewed in van
Lohuizen 1999) and MLL.+/+ mice suffer severe hema-
topoietic defects (Yu et al. 1995). We found that overpro-
duction of hemocytes in mxc larvae depends on the
transcriptional activator BRM. BRM is homologous to
yeast SWI2, a DNA-stimulated ATPase that is part of the
large SWI/SNF protein complex that modifies target
transcription by changes in chromatin structure (Tam-
In larvae, BRM activates HOM gene expression and is
required for imaginal disc cell viability (Elfring et al.
1998). BRM has many targets, so our results could reflect
a general requirement for BRM dosage on cell division.
Still, brm+/+ animals show no developmental delay and
loss of a brm+ copy had no effect on hematopoietic over-
proliferation induced by gain of Toll signal. Hence brm
could well have a positive part in blood cell number or
division control. brm could, as a trxG gene, be required
at the same level as but antagonistic to mxc. Alternatively,
since hemocyte phenotypes induced by loss of mxc and
by activation of JAK are both partially suppressed by loss
of brm, brm, mxc, and hop could all provide separate
regulatory inputs, which together control hematopoietic
cell divisions and cell density and cell survival in the
larva.

moira encodes a Drosophila homolog of human and
yeast chromatin-remodeling factors; mor and brm inter-
act genetically and MOR interacts physically with BRM
in the same large chromatin-remodeling protein com-
plex in the embryo (Crosby et al. 1999). Yet mor muta-
tions in trans had no effect on hematopoiesis regulation.
One possible explanation might be that composition of the
SWI/SNF protein complex is different in embryos
and in the lymph glands. Alternatively, as previously
argued for the PcG, haploidy for mor (or for trx) may
not reduce gene products enough to cause a mutant
phenotype.

We found that three genes, mxc, brm, and dom, required
for maintenance of HOM gene expression patterns are
involved in control of Drosophila hematopoiesis. dom,
like brm, encodes SWI2/SNF2 family DNA-dependent
ATPases involved in gene expression control through
modulation of chromatin structure (Ruhf et al. 2001).
Interestingly, hypomorphic dom phenotypes indicate
that dom, like mxc, negatively regulates lymph gland cell
proliferation and maintains lymph gland cell identities
(Braun et al. 1997, 1998; Ruhf et al. 2001), whereas brm
activates proliferation. These effects recall dom and mxc
repression and brm activation of HOM gene expression
(Ruhf et al. 2001), suggesting the possibility that in the
lymph glands, dom, mxc, and brm could participate in a
common mechanism of proliferation and identity main-
tenance, which could involve modulation of chromatin
structure.

mxc controls blood cell proliferation and differentia-
tion but not lineage specification: We have shown that
mxc functions as a cell autonomous regulator of cell
divisions in the lymph glands, as well as in circulating
blood cells, and that loss of mxc favors differentiation
of plasmatocyte lineage-specific cells such as podocytes
and lamellocytes. All blood cell types found in mxc mu-
tants, even podocytes, are found in wild type.
cells, although fewer, were always present in mxe mutants. We interpret this as meaning that loss of mxe does not change hemocyte lineage specification in the lymph glands. Rather, mxe controls steady-state hemocyte numbers in the body cavity of the larva. mxe and brn could both provide regulatory inputs in this process, together with domino, the JAK and Toll pathways, and other products, including the cell cycle regulated Pendulin protein, the Drosophila homolog of the mammalian S6 riboprotein, and the l(3)mbn-encoded plasma membrane protein (reviewed in Dearolf 1998). Together our data indicate that wild-type mxe product in larval hematopoiesis would maintain the normal rates of plasmatocyte proliferation and of crystal cell formation, as well as the normal timing of differentiation into self-recogizing macrophages. Under this hypothesis, mxe function in hematopoiesis would still be similar to PcG function in segmental identity specification in that both ensure that normal structures develop at the right time and place.

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LITERATURE CITED


