A role for the *Drosophila* Toll/Cactus pathway in larval hematopoiesis

Ping Qiu¹, Pang Chu Pan¹,² and Shubha Govind¹,²,*

¹Biology Department, City College and ²The Graduate Center, City University of New York, 138th Street and Convent Avenue, New York, NY 10031, USA

*Author for correspondence (e-mail: sgovind@scisun.sci.ccny.cuny.edu)

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SUMMARY

In the *Drosophila* larva, blood cells or hemocytes are formed in the lymph gland. The major blood cell type, called plasmatocyte, is small, non-adhesive and phagocytic. Plasmatocytes differentiate into adhesive lamellocytes to form multilayered capsules around foreign substances or, in mutant melanotic tumor strains, around self tissue. Mutations in *cactus* or *Toll*, or constitutive expression of *dorsal* can induce lamellocyte differentiation and cause the formation of melanotic capsules. As maternally encoded proteins, *Toll*, *Cactus* and *Dorsal*, along with *Tube* and *Pelle*, participate in a common signal transduction pathway to specify the embryonic dorsal-ventral axis. Using the maternal pathway as a paradigm, we investigated if these proteins have additional roles in larval hemocyte formation and differentiation. Analysis of *cactus* mutants that lack *Cactus* protein revealed that almost all of these animals have an overabundance of hemocytes, carry melanotic capsules and die before reaching pupal stages. In addition, the lymph glands of *cactus* larvae are considerably enlarged. The number of mitotic cells in the *cactus* and *Toll¹²* hemolymph is higher than that in the wild-type hemolymph. The hemocyte density of mutant *Toll, tube* or *pelle* hemolymph is significantly lower than that of the wild type. Lethality of mutant *cactus* animals could be rescued either by the selective expression of wild-type *Cactus* protein in the larval lymph gland or by the introduction of mutations in *Toll, tube* or *pelle*. *Cactus*, *Toll*, *Tube* and *Pelle* proteins are expressed in the nascent hemocytes of the larval lymph gland. Our results suggest that the *Toll/Cactus* signal transduction pathway plays a significant role in regulating hemocyte proliferation and hemocyte density in the *Drosophila* larva. These findings are discussed in light of similar hematopoietic functions of Rel/IκB-family proteins in mice.

Key words: Signal transduction, *Toll/Cactus*, NF-κB/IκB, Lymph gland, *Drosophila*

INTRODUCTION

In vertebrates, undifferentiated hematopoietic stem cells give rise to progenitor cells, which constitute the basis of several cell lineages. In each lineage, a number of temporally and spatially restricted developmental switches result in the production of mature cells. Some mature cells can be stimulated to undergo additional division and differentiation. In invertebrates such as *Drosophila*, hematopoietic stem cells are located in the lymph gland (Stark and Marshall, 1931; Rizki, 1978), and they give rise to progenitors of at least two lineages, plasmatocytes and crystal cells (Shrestha and Gateff, 1982).

Plasmatocytes are the predominant form of hemocytes in the wild-type larval hemolymph and, like mammalian macrophages or neutrophils, they perform phagocytic functions. Plasmatocytes are small, spherical and non-adhesive, and engulf bacteria and cell debris (Rizki and Rizki, 1980). Plasmatocytes also secrete extracellular matrix components (Fessler et al., 1994). When a larva experiences an immune challenge, plasmatocytes become stimulated, increase in number and, depending on the nature of infection, engage in phagocytosis or differentiate into discoidal and adhesive lamellocytes (Rizki and Rizki, 1992). Lamellocytes do not show any capacity for phagocytosis. Instead, they form multilayered capsules around foreign invaders or objects that are too large for phagocytosis (Rizki and Rizki, 1980, 1984). These capsules get melanized by activities of crystal cells. Crystal cells house the substrates and enzymes for melanization reactions (Rizki, 1957; Rizki and Rizki, 1980; Shrestha and Gateff, 1982).

In the absence of an immune challenge, plasmatocytes of a normal larva differentiate into lamellocytes at the onset of pupariation (Rizki, 1957). However, in certain *Drosophila* mutants, lamellocytes form melanotic capsules around self tissue, even in the absence of an immune challenge. The mechanisms that control the production, differentiation and functions of these cells in wild-type and mutant *Drosophila* are poorly understood.

Numerous studies performed on mammalian cells and in mice have underscored the important role that NF-κB/IκB-family proteins play in immune cell formation, differentiation and function (reviews Baeuerle and Baltimore, 1996; Baldwin, 1996). An indication that NF-κB/IκB-family proteins in *Drosophila* may also regulate hemocyte-related functions comes from observations that melanotic capsules composed of lamellocytes are found in the *Drosophila* IκB protein Cactus-deficient animals (Roth et al., 1991). Furthermore, constitutive
mutations in the *Drosophila* Toll receptor, or an overexpression of NF-κB/Rel-family transcription factor Dorsal in larvae also cause the formation of melanotic capsules (Gerttula et al., 1988; Govind, 1996). Toll, Cactus and Dorsal proteins were first described to function together in a maternally encoded signal transduction pathway that plays a central role in the specification of the dorsal-ventral (d/v) polarity of the *Drosophila* embryo. Melanotic encapsulations observed in *cact*, *TPD* and *hsp83dl* animals suggested to us that these proteins function in an unidentified hemocyte-related process, which is potentially important in the cellular immune response or normal development of *Drosophila* (Govind, 1996).

During early embryogenesis, *cactus* (*cact*), *dorsal* (*dl*), *Toll* (*Tl*) and six other genes [gastrulation defective (*gd*), snake (*snk*), *easter* (*ea*), *spätzle* (*spz*), *tube* (*tub*) and *pelle* (*pll*)] are required for d/v axis formation (review Belvin and Anderson, 1996; Drier and Steward, 1997). These genes are transcribed during oogenesis. In the syncytial blastoderm embryo, the gene products act in a stepwise manner to generate intracellular asymmetry along the d/v axis, which eventually generates d/v asymmetry in the developing organism. Three genes, *gd*, *snk* and *ea* encode serine proteases (Chasan and Anderson, 1989; DeLotto and Speirer, 1989; unpublished information of Konrad and Marsh, 1990), whereas *spz* encodes a growth factor-like molecule (Morisato and Anderson, 1994). These four proteins are secreted into the perivitelline fluid, where the serine proteases act in a cascade to process Spätzle. Activation of Spätzle occurs in a ventrally restricted manner, and active Spätzle serves as the ligand for the transmembrane Toll receptor on the ventral and lateral sides of the embryo, even though Toll is uniformly distributed (Hashimoto et al., 1991; Schneider et al., 1994; Morisato and Anderson, 1994).

The ventrally restricted activation of Toll is transduced by the cytoplasmic proteins Tube and Pelle. Structurally, Tube is a unique protein (Letou et al., 1991), whereas Pelle is a serine/threonine kinase (Shelton and Wasserman, 1993). The Toll/Tube/Pelle signal then acts on a non-covalent complex of Dorsal and Cactus proteins. Cactus has strong affinity for Dorsal, an interaction that causes the retention of Dorsal in the cytoplasm (Kidd, 1992; Whalen and Steward, 1993). The Toll/Tube/Pelle signal mediates phosphorylation and degradation of Cactus and the concomitant nuclear translocation of Dorsal (Belvin et al., 1995). The process of nuclear localization of Dorsal is graded, so that nuclei in the ventral-most position have the highest concentration of Dorsal, whereas those present laterally have lower amounts of Dorsal (Steward et al., 1988). Thus, after signaling, Cactus can be visualized in a dorsal-to-ventral cytoplasmic gradient, which inversely correlates with the ventral-to-dorsal gradient of nuclear Dorsal protein (Bergmann et al., 1996; Reach et al., 1996).

All seven genes that promote the nuclear localization of Dorsal belong to the ‘dorsal group’ of genes (Anderson and Nüsslein-Volhard, 1986). In embryos derived from females mutant for these seven genes, the nuclear localization of Dorsal is disrupted; Dorsal remains complexed to Cactus in the cytoplasm, embryos develop abnormally and become completely dorsalized (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989). Females carrying mutations in *cact* result in ventralization of the embryo (Roth et al., 1991). In these embryos, Dorsal is imported into nuclei along the dorsal and ventral sides (Roth et al., 1989; Steward, 1989).

In addition to their function in the d/v pathway, Spätzle/Toll/Tube/Pelle/Cactus are also required for proper activation of the antifungal peptide gene *drosomycin* in the adult fat body. *drosomycin* transcription is silent in uninfected animals, but its expression is induced by immune challenge. However, in *spz*, *Tl*, *tub* or *pll* mutants, *drosomycin* cannot be fully activated. In contrast, in mutant *cact* animals, *drosomycin* is transcriptionally active, even in the absence of an immune challenge (Lemaître et al., 1996). This immunity function of Cactus is genetically independent of Dorsal, even though Dorsal is expressed in the fat body (Lemaître et al., 1995). Instead, the partner of Cactus is believed to be the Dorsal-related immunity factor, Dif (Ip et al., 1993). Like Dorsal, Dif is found in the cytoplasm of fat body cells and interacts with Cactus (Lehming et al., 1995; Tatei and Levine, 1995). The relocalization of both these Rel proteins to the nucleus is affected by an immune challenge (Ip et al., 1993; Lemaître et al., 1995).

Signaling through Toll and Cactus proteins and activation of the Dorsal morphogen parallels signaling induced by Toll/IL-1 receptor (IL-1R) activation in mammalian cells: Toll/IL-1R and IkB signal the activation of NF-κB, the transcription factor that controls the transcription of many genes that mediate inflammation and other immune-related functions (Baldwin, 1996). The *Drosophila* and human Toll receptors are remarkably well conserved (Medzhitov et al., 1997). In addition, the cytoplasmic domains of both the *Drosophila* and human Toll proteins are homologous to the cytoplasmic domain of IL-1R (Schneider et al., 1991). Dorsal/NF-κB and Cactus/IκB belong to distinct families of transcription factors and their inhibitors (Steward, 1987; Geisler et al., 1992; Kidd, 1992; Baldwin, 1996). Thus, Toll/IL-1R receptor-dependent activation and nuclear localization of NF-κB-family transcription factors is a regulatory mechanism that is conserved in insects and mammals.

In this paper, we have explored the possibility that Toll, Cactus and Dorsal play a role in a hemocyte-dependent function in *Drosophila*. We analyzed the zygotic lethal phenotype of *cact* and found that absence of Cactus results in a highly penetrant overproliferation of hemocytes. This phenotype can be rescued by selective expression of wild-type Cactus in the nascent hemocytes of the mutant larval lymph gland. In genetic experiments, we report that lethality and melanotic encapsulation of *cact* are suppressed by mutations in *Tl*, *tub* and * pll*, but not by mutations in *spz*, *dl* or any of the other ‘dorsal group’ genes that are responsible for generating active Spätzle in the embryo. Based on these results, we propose that a zygotic Toll/Cactus pathway regulates hemocyte proliferation in the lymph glands during larval development and, in part, controls the steady-state number of circulating hemocytes.

**MATERIALS AND METHODS**

**Measurement of zygotic viability of *cactus* alleles**

The majority of *cact* stocks used in this study were obtained from the Nüsslein-Volhard laboratory and genotypes are listed on FlyBase (http://flybase.bio.indiana.edu/). Balanced chromosomes and phenotypic markers are described in Lindley and Zimm (1992) and on FlyBase. In antibody staining experiments, the P-element-induced *cact* and EMS-induced *cact* were found to be protein null alleles (Bergmann et al., 1996; Qiu and Govind, unpublished) and were used in crosses to measure zygotic viability. Viability was calculated by...
dividing the observed number of straight-winged (heteroallelic cact^+^) flies by the expected number of these flies. In the absence of lethality, the number of straight-winged flies is half of that of the curly winged heterozygotes. Based on the severity of the embryonic phenotype, the maternal-effect ventralization of cact has been previously classified into the strong (V2), intermediate (V3) and weak (V4) classes (Roth et al. 1991). We tested viability of 15 cact alleles that belong to these different classes and found that alleles that show the strongest maternal-effect phenotype also show the greatest loss of viability (1-35% of the expected progeny were viable). The intermediate V3 alleles were also semilethal (44-88% viability). The V4 alleles did not significantly compromise zygotic viability (92-100% viability). The direction of the semilethal (44-88% viability). The V4 alleles did not significantly compromise zygotic viability (92-100% viability). The direction of the semilethal viability.

**Analysis of the cactus phenotype**

The development of cact null (E8/D13), V2 (IIIG/E8), V3 (PD), V4 (Q6) and gain-of-function E10/E8 (previously crossed into yw; cact/CyO y^+^ background to identify mutants) larvae was followed every 24 hours after hatching. A 4-hour embryo collection was synchronized at the time of hatching. Melanotic capsules of cact^+^ larvae were fixed in 4% glutaraldehyde and were either stained with Hoechst or examined without staining.

**Rescue**

Cactus protein was expressed in specific tissues via UAS-cact/GAL4, a system that allows the selective activation of any cloned gene in tissue-specific patterns (Brand and Perrimon, 1993). Using the Nor1 and Kpm1 sites of the pUAST transformation vector (Brand and Perrimon, 1993), the zygotic cact cDNA was inserted 3' to the GAL4 UAS control element. Transfomant flies were obtained by microinjection, as described in Rubin and Spradling (1982), using a yw, A2-3 Sb/TM3 recipient strain. Ten different transformant lines were obtained. Line 82 (on chromosome II) and 100 (on chromosome III) were used in all experiments. Null cact^D13^/cact^S1^ and hypomorphic cact^D13^/cact^D13^ allele combinations were chosen for rescue. A series of GAL4 lines, whose tissue-restricted expression pattern was previously reported (Harrison et al., 1995), were obtained from Dr Perrimon’s laboratory and were used for driving the P[w^+^ UAS-cact^+^] transgene. The second chromosome insertion (P[w^+^ UAS-cact^+^82]) was recombined with cact alleles to obtain either the P[w^+^ UAS-cact^+^] b cact^2^CyO or the P[w^+^ UAS-cact^+^] b cact^D13^CyO stocks. Third chromosome GAL4 insertion lines were crossed into the b cact^D13^CyO background. Likewise, the second chromosome GAL4 insert was recombined with cact^D13^ chromosome for the reciprocal cross with third chromosome (P[w^+^ UAS-cact^D100^] insertion, previously crossed into the cact^+^ background. The UAS and GAL4 stocks were crossed with each other to yield a heteroallelic cact^+^ combination. Rescued cact^+^ animals are black-bodied and dark-red-eyed with two w^+^ transgenes, and were distinguishable from cact^-^ siblings with either the P[w^+^ UAS-cact^+^] or P[w^+^ GAL4] transgene. Percentage survival of cact^-^ adults is calculated as a fraction relative to the average of the number of adults in the remaining six sibling classes.

**Counting hemocytes**

Larvae of the appropriate genotype were selected and the hemolymph was withdrawn with a pipetman and placed on a 5 µl drop of light mineral oil, already placed on a hemocytometer. A coverslip was placed on the hemolymph and oil drop. All hemocytes that occupied an area of 0.16 mm^2^ in a depth of 0.1 mm were counted. The number of straight-winged flies is half of that of the curly winged mutants.

**Antibody-staining experiments**

Lymph gland preparations were made from wandering third instar larvae that were dissected in PBS. For hemocyte staining, a smear was prepared by making a fine incision of similar larvae and allowing the hemolymph to dry briefly on the slide. Dissected lymph glands were allowed to briefly adhere to the slide and both these samples were fixed for 20 minutes in 3.7% formaldehyde in PBS. After 5 washes with PBS, the samples were blocked with 2% bovine serum albumin and then incubated with each of these antibodies: 1:1 dilution of each of mouse anti-Cactus, or mouse anti-Dorsal (recognizes a specific epitope in the C-terminal half of Dorsal; Whalen and Steward, 1993).

**Dominant interactions between cactus and dorsal group genes**

The dominant class I Toll alleles (Schneider et al., 1995, T1P^+) used in this study are T1P^0^ ru ca/T1;3;OR60/TM3 and T1P^+^ e mwh/T1;3;OR60/TM3 and were obtained from K. Anderson. To check for the dominant interactions between T1 and cact, double mutant flies cact^BQ^/cact^D13^; T1P^+^/+ were constructed as follows: cact^BQ^ was crossed into CyO and TM3 double balanced background. Then b cact^BQ^/CyO; +/TM3 Sb were crossed with T1P^3^/TM3 males. In the next step, b cact^BQ^/T1P^3^ Sb males were crossed with b cact^D13^ CyO to obtain b cact^BQ^/b cact^D13^; T1P^+^/+ flies. Flies of all genotypes from the last cross were sorted and counted. The presence of melanotic capsules, appearing as dark spots mostly on the ventral side of the adult, was documented.

**Construction of the double mutants appearing between cactus^A2^ and dorsal group genes**

The viability of heteroallelic snk, ea, spc, Ti, plh and homozygous gd and tub combination with homozygous cact^A2^ was examined. The loss-of-function mutant stocks for dorsal group genes st snk^D12^ e/TM3, B^2^ B; y; ru st snk^29^ e ca/TM3 Sb, st ea^46^ e/TM3 Sb, mwh red e plh^86^/TM1 (obtained from Nüsslein-Volhard lab), ea^2^ et STm3, e spc^19^ ca/TM1, mwh red e spc^60^/TM1, TiP^4^ st e/TM3 Ser e. Df(3R)ro^80^ st e/TM3, tub^238^ st e/TM3, plh^11^ ru ca ca/TM3 Sb (obtained from K. Anderson) are described in Schneider et al. (1991). Roth et al. (1991) and Hecht and Anderson (1993). Mutants carrying dorsal group mutations on the third chromosome are marked with ebony, as is the TM3 Sb balancer. To construct the double mutant flies, stocks of the above genotypes were crossed with w^+^; Sp/CyO; CyO/TM3 Sb (obtained from P. Szibere). The dorsal group/TM3; Sp/+ males were backcrossed to obtain individual Sp/CyO: dorsal group/TM3 stocks. These were then crossed with the A2/Cyo; +/TM3 males to yield cact^D12^/CyO; dorsal group/TM3 stocks. For mutations on the third chromosome, the expected ratio of double heterozygotes: single homozygous (2 classes): double homozygous is 4:2:2:1. For mutations on the first chromosome, (gd), this ratio of the surviving progeny is 2:1:1 and for the second chromosome mutations (df), the expected ratio of the balance class to double homozygotes is 2:1. Percentage viability of double homozygotes and single homozygotes was calculated relative to the double balanced class. Double balanced stock gd^2^ v FM3; b cact^A2^ ec/CyO and b cact^A2^ df/CyO were obtained from Y. Hiromi and S. Roth.
RESULTS

cactus null animals are developmentally arrested

To identify the lethal phase of cact mutants, we examined the null (cact<sup>E8</sup>/cact<sup>D13</sup>) and hypomorphic (cact<sup>E8</sup>/cact<sup>IIIG</sup>) larvae through different larval and pupal stages. These larvae are viable at 24 and 48 hours after fertilization (Fig. 1A,B). By 72 hours, there is some larval lethality and delay in the rate of development of the null animals when compared to their heterozygous siblings (Fig. 1A). A clear difference between null and hypomorphic animals in their viability and melanotic capsule phenotype becomes evident by 120 hours. Whereas only 40% of the expected cact null animals survive to 120 hours, more than 80% of the expected hypomorphic animals are alive (Fig. 1A,B). In the following days, this difference is even more pronounced: only 4 out of 100 cact null larvae make white prepupae; the remaining animals persist as larvae and eventually die (Fig. 1C). In contrast, more than 80% of the hypomorphic progress into pupal stages (Fig. 1C). However, less than 1% of these animals eclose, while the majority die as pupae.

To determine if the incidence of encapsulation in cact animals correlates with zygotic lethality and if capsules are present in null larvae, we examined larvae of different allele classes for the presence of dark spots. Neither larvae nor adults of the V4, V3 or gain-of-function cact classes (see Materials and Methods) have melanotic capsules. In contrast, about 40% of the hypomorphic larvae and almost all of (over 90%) the null larvae bear melanotic capsules (Figs 1D, 2A). The high penetrance of capsule formation in cact null animals and the strong correlation of encapsulation with lethality suggest that encapsulation of self tissue is at least one of the primary zygotic phenotypes of cact.

Hematopoietic defects of cactus

To identify the tissues or organs where cact function may be required, we examined capsules and accompanying tissues from mutant larvae. Aggregates of hemocytes are often found in association with the larval fat body (Fig. 2B). Melanization in the cact capsules appears to initiate at discrete spots (Fig. 2E). As larvae grow, the size of capsules and the area of the melanized foci become larger (Fig. 2F). Cells of the cact fat body show variable loss of intercellular adhesion. In addition to these defects, salivary glands appear atrophied and sometimes show...
melanization that is not accompanied by hemocytic encapsulation. These phenotypic defects are strikingly similar to those observed when Dorsal is overexpressed (Govind, 1996).

To determine if the high incidence of melanotic capsules in cactus mutants is caused by an increase in hemocyte concentration, by increased hemocyte differentiation, or by both these processes, we compared the hemocyte concentration and differentiation in mutants and heterozygotes. The number of hemocytes/μl in cact hemolymph is more than ten-fold higher than in the hemolymph of heterozygous siblings or wild-type larvae (Figs 2C,D, 3). However, hemocyte density in the gain-of-function cact larvae (which do not show presence of capsules) is not significantly different from the heterozygous or Canton-S control (Fig. 3). We also determined the ratio of plasmatocytes to lamellocytes in cact hemolymph in a genetic background that marks lamellocytes, but not plasmatocytes. A P-lacZ enhancer trap line (P1587) was crossed into the cact E8/cact D13 background. When hemocyte smears were stained for β-galactosidase activity, we found that roughly one quarter (537/2244) of all hemocytes in circulation turned blue. In contrast, hemocytes of the enhancer trap line (cact+ background) showed no detectable β-galactosidase activity. This estimate of lamellocyte number does not include those cells that are no longer in circulation and have been recruited to form capsules.

To determine if the overabundance of circulating hemocytes in cact animals is due to an oversized lymph gland, we...
Table 1. Rescue of cact⁻ lethality correlates with the expression of wildtype Cactus protein in the larval lymph gland

<table>
<thead>
<tr>
<th>GAL4 line</th>
<th>Lymph gland expression</th>
<th>Total progeny analyzed</th>
<th>UAS/GAL4 A2/D13</th>
<th>Average % Back ground (expected)</th>
<th>% increase in survival</th>
<th>Total progeny analyzed</th>
<th>UAS/GAL4 A2/D13</th>
<th>% increase in survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>e33C</td>
<td>strong, uniform</td>
<td>713</td>
<td>50</td>
<td>45</td>
<td>103</td>
<td>48.5</td>
<td>43.7</td>
<td>-</td>
</tr>
<tr>
<td>T100</td>
<td>weak, patchy</td>
<td>496</td>
<td>30</td>
<td>22</td>
<td>74</td>
<td>40.5</td>
<td>40.5</td>
<td>-</td>
</tr>
<tr>
<td>T98</td>
<td>weak, uniform</td>
<td>379</td>
<td>18</td>
<td>43</td>
<td>53</td>
<td>35.1</td>
<td>40.4</td>
<td>5.3</td>
</tr>
<tr>
<td>T59</td>
<td>weak, uniform</td>
<td>379</td>
<td>18</td>
<td>43</td>
<td>53</td>
<td>33.9</td>
<td>81.1</td>
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<tr>
<td>e33C</td>
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<td>40</td>
<td>74</td>
<td>77</td>
<td>51.9</td>
<td>96.1</td>
<td>44.2</td>
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</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of flies</th>
<th>No. of MT</th>
<th>% penetration</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>155</td>
<td>1</td>
<td>0.65</td>
</tr>
<tr>
<td>+/cactD13</td>
<td>256</td>
<td>110</td>
<td>43.0</td>
</tr>
<tr>
<td>cactBQ/cactD13</td>
<td>59</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Dominant interactions between Tl0 and cact⁻

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TP0/+ No. of flies</th>
<th>TP0/+ No. of MT</th>
<th>TP0/+ % penetrance</th>
<th>Tl0/+ No. of flies</th>
<th>Tl0/+ No. of MT</th>
<th>Tl0/+ % penetrance</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>155</td>
<td>1</td>
<td>0.65</td>
<td>176</td>
<td>1</td>
<td>0.57</td>
</tr>
<tr>
<td>+/cactD13</td>
<td>256</td>
<td>110</td>
<td>43.0</td>
<td>171</td>
<td>87</td>
<td>50.9</td>
</tr>
<tr>
<td>cactBQ/cactD13</td>
<td>59</td>
<td>0</td>
<td>0</td>
<td>38</td>
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<td>0</td>
</tr>
</tbody>
</table>

Dominant alleles Q and Tl0 of Tl were crossed into cactD13 and cactBQ/cactD13 backgrounds. cactD0 is a gain-of-function allele and its maternal effect causes dorsalization of embryos. The incidence of melanotic capsules in adults (MT) was recorded. Heterozygous cactD13 condition does not cause melanotic capsule formation or lethality.
Dorsal suggests that some of these same 'dorsal group' genes may play a role in larval hematopoiesis, and possibly act in the same pathway as Cactus. To determine whether 'dorsal group' and Toll proteins also act in a signal transduction pathway.

**Table 3. Viability of double mutants between cact^A2 and dorsal group genes**

<table>
<thead>
<tr>
<th>Parents</th>
<th>Genotypes of progeny</th>
<th>Expected no. of progeny (relative to double heterozygote)</th>
<th>Observed no.</th>
<th>% Viability (relative to double heterozygote)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/CyO: T^P244/TM3Sb</td>
<td>708</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>354</td>
<td>3</td>
<td>0.85</td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>354</td>
<td>239</td>
<td>67.5</td>
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<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>177</td>
<td>84</td>
<td>47.5</td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>959</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>480</td>
<td>2</td>
<td>0.42</td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>480</td>
<td>11</td>
<td>2.3</td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>240</td>
<td>115</td>
<td>47.9</td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>397</td>
<td>2</td>
<td>0.50</td>
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<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>397</td>
<td>132</td>
<td>33.2</td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>199</td>
<td>108</td>
<td>54.4</td>
</tr>
<tr>
<td>A2/CyO: T^P244/TM3Sb × A2/CyO: T^P244/TM3Sb</td>
<td>A2/A2; T^P244/TM3Sb</td>
<td>409</td>
<td>5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Based on the number of flies that contain one copy of each of the mutations and both balancers (the double balancer class), the expected number of single and double homozygotes is calculated (Methods and Materials). Percentage viability of the double homozygotes (cact^A2/cact^A2; dorsal group/dorsal group) and cact^A2/cact^A2; dorsal group/Balancer class is compared and graphically presented in Fig. 5. It is important to note that the effect of dorsal group genes on the cact^A2/cact^A2 phenotype is recessive. The percentage viability of the different ‘dorsal group’ gene stocks or transheterozygous combinations (in the absence of cact mutation) was also checked and was similar to that observed for the cact^A2/CyO; dorsal group/dorsal group class shown here (data not shown). Data presented here show the suppression of cact^A2 lethality by expression of Toll, tub and pII, but not by dl.

Since the degree of cact rescue is also not proportional to the level of Cactus expression in the fat body (for example, e13C is fat body positive for GAL4 expression, whereas e33C shows no expression in the fat body), it appears that the function of Cactus in the lymph gland is distinct from its function in the fat body, where it regulates activation of drosomycin and cercepin (Lemaitre et al., 1996). The hematopoietic defects observed in cact mutants and the rescue of cact lethality by expression of wild-type Cactus protein in the lymph gland strongly argue that the normal function of Cactus is important for regulating the process of hemocyte formation.

**Dominant interactions between cactus and Toll**

The evolutionary conservation in the structure and function of the intracellular compartment proteins Toll, Pelle, Cactus and Dorsal suggests that some of these same ‘dorsal group’ genes may play a role in larval hematopoiesis, and possibly act in the same pathway as Cactus. To determine whether ‘dorsal group’ gene products that act in the early embryo, also play a role in controlling Cactus function in the lymph gland, we tested for dominant and recessive genetic interactions between cact and ‘dorsal group’ genes.

To test for dominant interactions, we crossed T^P10^b and T^P9^ alleles (T^P^) into the cact^D13^+/+ or gain-of-function (cact^D13^/cact^BQ^) backgrounds and documented the penetrance of the melanotic capsules in adult flies (Table 2). Heterozygous cact adults do not show melanotic capsules, and less than 1% of the T^P/+ flies carry these capsules. However, when the dose of cact is lowered by introduction of the null cact^D13^ allele into the T^P/+ background, between 40 and 50% of the adults have melanotic capsules. On the contrary, in the presence of the gain-of-function Cactus^BQ^ protein, none of the adults show such capsules (Table 2). These genetic results suggest that, like their maternally expressed counterparts, zygotic Toll and Cactus proteins also act in a signal transduction pathway.

**Mutations in Toll, tube and pelle rescue cactus^A2^ lethality**

To ask if other ‘dorsal group’ genes also control the function of zygotic Cactus, we constructed double mutants between cact^A2^ and ‘dorsal group’ genes and measured the viability of resulting double mutant flies (Table 3; Fig. 5). We chose the cact^A2^ allele for construction of double mutants because of its low zygotic viability. Loss-of-function mutations in snk, gd, ea and spz did not affect the proportion of the homoygous cact^A2^ animals that survived to adulthood (<25%). Some variability was observed and most likely reflects differences in genetic background (Fig. 5). In contrast to these four ‘dorsal group’ genes, mutations in Tl, tub and pII strongly suppress cact^A2^ lethality. Viability of these double mutants increases from less than 1% to more than 45%, and melanotic capsules found in cact^A2^ single mutants are rarely observed in these double mutant adults. To test if mutations in dl, the gene that encodes the Rel partner of Cactus in the embryo can also modify cact^A2^ lethality, we examined the viability of cact^A2^ dl^d^ double mutants and found it to be in the same range as cact^A2^ homozygotes (Table 3; Fig. 5).

**Table 4. Mitotic cells in wildtype and mutant hemolymph**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Plasmatocytes</th>
<th>Dividing cells</th>
<th>% cells in division</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>1,585</td>
<td>16</td>
<td>1.01</td>
</tr>
<tr>
<td>cact^-/-</td>
<td>1,443</td>
<td>65</td>
<td>4.5*</td>
</tr>
<tr>
<td>T^P/+</td>
<td>2,458</td>
<td>21</td>
<td>0.85</td>
</tr>
<tr>
<td>T^P/+</td>
<td>2,327</td>
<td>44</td>
<td>1.9*</td>
</tr>
</tbody>
</table>

Hemolymph smears from ten or more larvae were stained in three or more independent experiments with an anti-phosphohistone H3 mitosis marker to visualize dividing cells. Number of hemocytes in circulation that showed mitotic figures (Fig. 7H) were counted, as were the number of plasmatocytes; lamellocytes do not divide and were, therefore, ignored. Using a comparison of proportions (Z-test), we found that values of wild-type and cact^-/-' and wild-type and T^P/+ samples were significantly (>95% confidence) different (indicated by asterisk), whereas those between wild-type and T^P/+ samples were not.
These genetic results are consistent with previous observations that mutations in \( gd, snk, ea, spz \) and \( dl \) do not compromise zygotic viability, whereas those in \( Tl, tub \) and \( pll \) cause the death of some animals (Gerttula et al., 1988; Hecht and Anderson 1993; Table 3). It appears that Toll, Tube and Pelle can modulate Cactus function that is important for larval or pupal viability. Since there is a direct correlation between \( cact \) lethality and the incidence of melanotic capsules, it is possible that these proteins modify Cactus function in the lymph gland and thereby suppress \( cact \) lethality. If Cactus protein is absent, then this effect of the Toll- Tube-Pelle signal on Cactus function is not measurable. We, therefore, performed hemocyte counts on \( Tl, tub, pll \) and \( tub \, pll \) mutants. Heterozygous siblings and wild-type larvae served as controls. As can be seen from Fig. 3, the concentration of hemocytes in these mutant combinations ranged from 21% to 48% of the wild-type concentration. Hemocyte concentration in \( Tl^{+} \) larvae is the lowest (1,222 cells/\( \mu l \)), as compared to 3,771 cells/\( \mu l \) of the heterozygous control hemolymph. In contrast, the hemocyte concentration in the \( Tl^{D} \) background is roughly two-fold (10,348 cells/\( \mu l \)) compared to the Canton-S hemolymph (5,523 cells/\( \mu l \)). When compared with the heterozygous controls, hemocyte concentration per microliter of \( tub \) (1,375 versus 6,381), \( pll \) (2,508 versus 5,142) or \( tub \, pll \) (1,875 versus 5,182) hemolymph also shows a statistically significant deficit (Fig. 3). In control experiments, we found that hemocyte concentration in unrelated semilethal/lethal strains is in the wild-type range (R. Sorrentino and S. G., unpublished data), suggesting that the effects of \( Tl, pll \) and \( tub \) mutations on hemocyte counts are particular to these mutations. Since \( Tl, tub \), and \( pll \) have phenotypic effects that are opposite of \( cact \), a deficit in hemocyte concentration in these three ‘dorsal group’ mutants is consistent with the genetic interaction data presented above. Together, these observations support the idea that a signal transduction pathway with Toll, Tube, Pelle and Cactus proteins is involved in controlling hemocyte density in the larval hemolymph.

**Toll, tube and pelle mutants have a hemocyte deficit**

A function for Cactus in the lymph gland lobes and the genetic suppression of \( cact \) lethality by mutations in \( Tl, tub \) and \( pll \) predicts that mutations in these latter genes should also cause hematopoietic defects. To determine if there are any obvious defects in the morphology of mutant lymph glands, we examined glands from \( Tl, tub, pll \) and \( tub \, pll \) larvae and did not find any apparent or striking differences in the size or the organization of lobes or pericardial cells (results not shown). We, therefore, performed hemocyte counts on \( Tl, tub, pll \) and \( tub \, pll \) mutants. Heterozygous siblings and wild-type larvae served as controls. As can be seen from Fig. 3, the concentration of hemocytes in these mutant combinations ranged from 21% to 48% of the wild-type concentration. Hemocyte concentration in \( Tl^{+} \) larvae is the lowest (1,222 cells/\( \mu l \)), as compared to 3,771 cells/\( \mu l \) of the heterozygous control hemolymph. In contrast, the hemocyte concentration in the \( Tl^{D} \) background is roughly two-fold (10,348 cells/\( \mu l \)) compared to the Canton-S hemolymph (5,523 cells/\( \mu l \)). When compared with the heterozygous controls, hemocyte concentration per microliter of \( tub \) (1,375 versus 6,381), \( pll \) (2,508 versus 5,142) or \( tub \, pll \) (1,875 versus 5,182) hemolymph also shows a statistically significant deficit (Fig. 3). In control experiments, we found that hemocyte concentration in unrelated semilethal/lethal strains is in the wild-type range (R. Sorrentino and S. G., unpublished data), suggesting that the effects of \( Tl, pll \) and \( tub \) mutations on hemocyte counts are particular to these mutations. Since \( Tl, tub \), and \( pll \) have phenotypic effects that are opposite of \( cact \), a deficit in hemocyte concentration in these three ‘dorsal group’ mutants is consistent with the genetic interaction data presented above. Together, these observations support the idea that a signal transduction pathway with Toll, Tube, Pelle and Cactus proteins is involved in controlling hemocyte density in the larval hemolymph.

**Mutations in Toll and cactus affect the number of cells undergoing division**

To investigate the cause of aberrant hemocyte concentrations in \( Tl \) and \( cact \) larvae, we examined if the number of hemocytes
in division is altered in these mutants. Dividing hemocyte precursors within lymph gland lobes as well as mature hemocytes in circulation have been observed by light and electron microscopy (Rizki, 1957; Shrestha and Gateff, 1982). Using an anti-phosphohistone H3 mitosis marker, which specifically stains all cells in division (Lu et al., 1994; Chadee et al., 1995), we found that the number of cells in division in the cact lymph gland is clearly higher than in the wild type (Fig. 6). Because of the small size of the hemocyte precursors within the lymph gland, mitotic patterns could not be quantified with the DAB staining technique. We, therefore, examined dividing hemocytes in circulation.

Feulgen stain was previously used to examine the fluctuations in cell division during larval development (Rizki, 1957). The first peak in the number of cells in division occurs 3 hours before the first molt. Second and third peaks coincide with the first and the second larval molts. About 10 hours later, the number of cells in division drops and remains low (1-2%) for the remainder of larval life. In our experiments, the mitosis marker was used to quantify hemocyte division in wild type, cact and Tl mutants during the late larval period when hemocyte division remains low. Consistent with the previous results, we found that the number of dividing hemocytes in wild-type hemolymph is 1.02%. This number is significantly higher in cact (4.5%) and the Tl+/+ (1.9%) backgrounds (Table 4; Fig. 7H). In contrast, the fraction of cells in division in Tl- hemolymph is slightly, but not significantly, lower (0.85%) than in the wild type.

**Expression of d/v proteins in larval lymph gland**

The cact phenotype, its rescue by Cactus expression in the lymph gland and the genetic suppression by mutations in Tl, tub and pll imply that Toll, Tube and Pelle proteins very likely convey an intracellular signal to Cactus within hemocyte precursors. If this is indeed the case, then each of these proteins is expected to be present in these cells. Using specific antibodies against these proteins, we examined the expression of Toll, Tube, Pelle and Cactus in larval lymph glands, and found that, consistent with the rescue results, all of these proteins are expressed in the hemocyte precursors, but not in the pericardial cells (Fig. 7B,E-G). In control experiments, where the primary antibody was omitted, no staining was observed (Fig. 7A). Application of anti-Dorsal antibody (Fig. 7C), or anti-Dif antibody (Fig. 7D) also revealed the presence of Dorsal and Dif proteins in lymph gland lobes, but not in the pericardial cells.

**DISCUSSION**

**Cactus function is important for larval hematopoiesis and it acts in a common pathway with Toll, Tube and Pelle**

Melanotic encapsulation of self tissue in almost all null cact larvae provided the first important clue regarding the biological applications of Cactus in hematopoiesis. The phenotypes of cact mutant larvae revealed the importance of Cactus function in this process. The rescue of cact phenotypes by Cactus expression in the lymph gland indicates that Cactus acts in a common pathway with Toll, Tube and Pelle. This pathway is likely mediated by intracellular signaling pathways that converge on Cactus within hemocyte precursors. The expression of these proteins in the hemocyte precursors, but not in the pericardial cells, suggests that the signaling pathway is specific to hematopoiesis.

**Fig. 6.** Division of nascent hemocytes in lymph glands from third instar larvae. (A,B) Wild-type lymph glands; (C) cact lymph gland. Preparations in B and C were stained with anti-histone H3 antibody, whereas preparation in A was not treated with this primary antibody. Arrows point to lymph gland lobes; P refers to enlarged pericardial cells of the mutant lymph gland.

**Fig. 7.** 5-day-old wild-type lymph glands stained with no primary antibody (A), anti-Cactus (B), anti-Dorsal (C), anti-Dif (D), anti-Toll (E), anti-Tube (F), anti-Pelle (G) antibodies. Expression is in nascent hemocyte cells of the lobes but not in pericardial cells (arrow B,C,D). All 100×, except E and F, 200×. (H) Anti-histone H3 antibody staining of hemocyte from Tl+/+ hemolymph. Mitotic figures are clearly visible (arrows).
function of this protein. The findings that *cact* hemolymph has about 10-fold more hemocytes and that the lymph glands of mutant animals are enlarged suggested that *cact* is a hematopoietic mutant. This suggestion was confirmed by the rescue of *cact* lethality upon selective expression of wild-type Cactus protein in the nascent hemocytes of the mutant lymph gland lobes. The GAL4 lines used in this study allowed us to distinguish the lymph gland function of Cactus from its function in the fat body, where it regulates the immune activation of antimicrobial genes (Lemaitre et al., 1996). The result that *cact* lethality cannot be rescued by expression of wild-type Cactus in the fat body also indicates that this function of Cactus in the activation of humoral immunity genes is not vital for development.

Rescue of *cact* lethality was also achieved by the introduction of mutations in *Tl, tub* and *pll* genes. Because of the reciprocal relationships of these genes (‘dorsal group’ gene products promote nuclear localization of Dorsal in the embryo; Cactus inhibits this process), we anticipated that those ‘dorsal group’ genes that interact with *cact* zygotically should act as suppressors, and not enhancers, of *cact* lethality. This expectation was borne out. The finding that mutations in *Tl, tub, pll* and *cact* show alterations in their hematocyte concentration and that each of these gene products is expressed in hematocyte precursors suggests that these four proteins function in a common pathway (Fig. 8), as they do in other processes in *Drosophila*. In addition to their functions in dorsal-ventral patterning and the induction of antimicrobial genes, Toll, Tube and Pelle also play a role in muscle patterning events in the larva (Halfon and Keshishian, personal communication). Furthermore, mutations in these three ‘dorsal group’ genes and *cact* also affect pupal shape and morphology (Letou et al., 1991). An additional role for Cactus in plasmatocyte differentiation and encapsulation is also possible and remains to be investigated.

Our genetic data suggest that *spz*, the gene that encodes the ligand for the maternal Toll, is not involved in Toll activation for hematocyte precursors. *spz* function has also been defined in the fat body and in muscle patterning (Lemaitre et al., 1996; Halfon and Keshishian, personal communication). Precedents exist for individual receptors that are involved in different biological functions to be activated by different ligands or combination of ligands. The *Drosophila* EGF receptor, involved in different signaling events (embryonic and imaginal disc development), is known to be activated by Gurken and Spitz (Rutledge et al., 1992; Neuman-Silberberg and Schüpbach, 1993). It may be that Toll similarly has multiple ligands in *Drosophila*, possibly tailored for its different functions. Consistent with an expectation of a new Toll ligand is the finding that gene products whose activities are required for activation of Spätzle during early embryogenesis (Gastrulation Defective, Snake and Easter), also do not modify *cact* lethality.

Like *spz, dl* is not expected to play a role in the Toll/Cactus hematopoietic pathway, even though ubiquitous Dorsal expression in the larva causes melanotic encapsulation and lethality. It is possible that Dif or a Dorsal-Dif heterodimer serves this function, given that both Dif and Dorsal proteins are expressed in the larval lymph gland. Furthermore, both Dorsal and Dif interact with Cactus (Kidd, 1992; Whalen and Steward, 1993; Lehning et al., 1995; Tatei and Levine, 1995) and, like the p50-p65 heterodimer of NF-kB, Dorsal and Dif can form heterodimers (Gross et al., 1996). Physiological evidence for such a function will come from analysis of *Dif/Dif dl* mutants.

**Role of Toll and Cactus in cell division**

Staining experiments with the mitosis marker show that the number of dividing hemocytes in the *cact* lymph gland is substantially higher than in the wild-type background (Fig. 6). Consequently, the lymph gland lobes are enlarged; more hemocytes are released from the mutant than from the wild-type lymph glands, and mutant hemocytes continue to divide at a higher rate even after their release into the hemolymph (Table 4; Fig. 7H). This result suggests that the normal function of Cactus is to modulate cell division by preventing overproliferation. Furthermore, constitutive activation of Toll also promotes cell division within nascent hemocytes, as the fraction of dividing hemocytes is also somewhat higher in *Tl/+* hemolymph.

While Toll activation promotes cell division, the absence of Toll function does not significantly reduce the number of dividing hemocytes, even though hematocyte density is markedly lower in *Tl* mutants. Similarly, the moderate increase (less than 5-fold) in hematocyte division does not measure up to the excessive increase (more than 10-fold) in hematocyte density in *cact* animals. These discrepancies suggest that the Toll/Cactus pathway exerts additional effects, either on the release of mature hemocytes from the lymph gland or on their survival after their release. It is worth noting that activation of NF-kB has been shown to potentiate apoptotic effects of tumor necrosis factor (Beg and Baltimore, 1996; Wang et al., 1996; Van Antwerp et al., 1996), and it is possible that the Toll/Cactus pathway also plays a role in controlling hematocyte viability in *Drosophila*.

**Conservation of Toll/Cactus pathway functions in development and immunity**

Alterations in hematocyte density in *Tl, tub, pll* and *cact* mutants suggest that the effects of this signal transduction pathway in hematopoiesis are limited: these genes do not play a role in hematocyte lineage specification. Instead, they appear to regulate the number of hemocytes in the body cavity so that there is an...
optimum range of steady-state hemocyte density during larval development. The nature of the signal that controls the activation of this Toll/Cactus pathway is not known. However, it is reasonable to expect that in an unchallenged animal, hemocyte proliferation, both in the lymph gland and in the hemolymph, is developmentally controlled. There is a transient increase in hemocyte density after an immune assault (Rizki and Rizki, 1992), and it is likely that this Toll/Cactus pathway plays a similar role in calibrating such a fluctuation in hemocyte density.

Since effects of the Toll/Cactus pathway mutants appear to be confined to the regulation of hemocyte density in the larva, other basal and regulatory signals must contribute more directly to lineage specification, hemocyte turnover and differentiation. Phenotypes of other hematopoietic mutants in Drosophila range from the absence of lymph glands to the presence of massively overgrown lymph glands (Gateff, 1978; Watson et al., 1991; Gateff, 1994). Analysis of some of these mutants suggests that these genes play specific roles in hemocyte specification, differentiation or turnover. For example, recent studies on the constitutive JAK mutant hop 
atl (which results in lymph gland overgrowth and hematopoietic neoplasm) suggest that, like the Toll/Cactus pathway, the JAK/STAT signaling pathway also regulates hematopoiesis and hemocyte density in the Drosophila larva (Harrison et al., 1995; Luo et al., 1995; Luo and Dearolf, 1997). It is possible that multiple regulatory inputs, such as the Toll/Cactus and JAK/STAT signals, are integrated with, or superimposed on one another, to ensure that hematopoietic precursors survive and divide, and progress normally through their developmental program to give rise to mature hemocytes in a consistently controlled manner.

The hematopoietic function of the Toll/Cactus pathway in Drosophila development and immunity appears to be conserved in mammals. Gene knockout experiments in mice show that the absence of specific NF-κB/IκB family proteins does not alter the specification of the various lineages. Rather, individual lineages are differentially affected in mutants. For example, mice deficient in the IκBα protein have a significantly elevated number of granulocyte (neutrophil) precursors and they exhibit neonatal lethality (Beg et al., 1995). relB- mice show an increase in the number of erythroid precursors in the spleen, but a decrease in erythroid precursors in the bone marrow (Weit et al., 1995). Mice lacking the c-rel proto-oncogene show normal numbers of hematopoietic cells, but lymphocytes in these animals show proliferative and immune-effecter defects that suggest that c-rel is crucial for cell division and immune function (Kontgen et al., 1995). These parallel phenotypes in flies and mice suggest that NF-κB/IκB functions ensure that hematopoietic cells proliferate in a controlled manner, while simultaneously guarding against production of excess daughter cells. When such mechanisms are perturbed, malignancies and abnormal genetic conditions manifest. The hematopoietic defects observed in cact mutants are reminiscent of lymphoid malignancies associated with mutations in vertebrate rel (v-rel and lyr-10) genes (Ballard et al., 1990; Neri et al., 1991). It is, therefore, possible that the NF-κB/IκB and Toll/Cactus pathways play very similar biological functions in distantly related animals, potentially through similar cellular and molecular mechanisms. The simpler hematopoietic lineage of Drosophila can serve as a model system to further our understanding of how NF-κB/IκB family proteins regulate hematopoietic and immune-related functions in vivo.

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