PROGRESS IN NUCLEIC ACID RESEARCH AND MOLECULAR BIOLOGY

VOLUME 82

EDITED BY
P. MICHAEL CONN

PROGRESS IN

Nucleic Acid Research and Molecular Biology

edited by

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Oregon Health and Science University
Beaverton, Oregon

Volume 82

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32 Jamestown Road, London, NW1 7BY, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

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British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-374549-1
ISSN: 0079-6603

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Printed and bound in the USA
08 09 10 11 12 10 9 8 7 6 5 4 3 2 1

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Drosophila Orthologues to Human Disease Genes: An Update on Progress

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Modeling human disease in flies is possible because many basic processes of cellular proliferation, motility, regulation and interaction are highly conserved among multicellular organisms. Despite years of extensive study, a clear understanding of the basic biology of many human illnesses still remains elusive. In part, this is due to a deficit in adequate genetic model systems to study pathogenesis of disease dynamics in a developing organism. *Drosophila* melanogaster is emerging as a model of choice to study the molecular genetic underpinnings of human disease. It should be noted as well that the selection of *Drosophila* to model human genetic disease is not only based on homology, but also on the wide variety of tools and a century of classic genetics that provide outstanding experimental capabilities. Recent advances in methodology have increased the value of this model system to study the basic science of human disease and opened up new opportunities. The elucidation in flies of the underlying regulated mechanisms of human disorders may eventually reveal new therapeutic targets for the treatment of diseases. Here we describe recent advances in the study of neurological disorders, blood diseases and even cancer. We also outline future directions in research on modeling many devastating diseases in the fruit fly *Drosophila melanogaster*.

Progress in Nucleic Acid Research and Molecular Biology, Vol. 82
DOI: 10.1016/S0079-6603(08)00001-9

0079-6603/08 $35.00

I. Introduction

The goal of this chapter is to present to a broader view of the state of human disease modeling in *Drosophila melanogaster* and to outline new directions in the study of the genetic basis of human disorders in flies. The *Drosophila* classical genetics powerhouse in combination with rapidly developing genomic and postgenomic tools accelerates the identification and characterization of gene networks. Because the molecular mechanisms controlling a variety of physiological pathways are largely conserved between flies and humans, flies are quite useful in modeling a variety of human diseases. These include nervous system disorders, cancer, immune responses, elements of the cardiovascular system, and many more (*1*). In addition, *Drosophila* genetic tools can also be used to study systems that are not evolutionally conserved or common between flies and humans. In fact, fly genetics has been applied to the dissection of certain basic metabolic pathways in human organs that are not even present or undeveloped in flies. Due to obvious anatomical differences, the humble fruit fly certainly will never compete with mammalian models in every aspect of human diseases research, but a century of fly genetics should not be underestimated. As a genetic model organism, *Drosophila* has much to offer human disease researchers in terms of genetic screening power, a wide variety of molecular tools, multiple stock centers packed with a variety of allele, transgene, and deficiency collections and at the same time, any fly geneticist will tell you that they offer an elegant simplicity that drives basic research discoveries even in inexperienced student investigators.

II. Neurological Disease

In terms of modeling human genetic disease in *Drosophila*, neurological diseases have been the most lucrative [reviewed in (*2*, *3*)]. This is not surprising considering a significant level of sequence and function conservation of nervous system genes and pathways that are directly relevant to human neurological disease [links between human disease and fly genes can be found using the Homophila database at http://homophila.sdsc.edu; (*1*, *4*)]. Although the basic processes of neurogenesis, neuronal pathfinding, and synaptogenesis have been studied in *Drosophila* for some time, recently, there has been a boost in *Drosophila* research focusing directly on models for neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, spinocerebellar ataxias, and Huntington's disease. These efforts have not only contributed to a better

understanding of the underlying basic genetic and molecular mechanisms of these disorders but also opened new avenues for practical pharmacotherapy and potential drug screening.

Contemporary genetic studies imply misexpression of the gene for *α-synuclein* in familial forms of Parkinson's disease. The common symptoms of this locomotion disorder are the presence of pathological aggregates of α-synuclein into inclusions known as Lewy bodies accompanied by the loss of dopaminergic neurons in substantia nigra (*5–7*). Despite the fact that there is no endogenous *α-synuclein* in flies, *Drosophila* models of Parkinson's disease have been created through transgenic expression of wild type or mutant forms of the human *α-synuclein* gene in flies. Gain-of-function expression of *α-synuclein* in the fly brain leads to Parkinson's pathology, recapitulating several important aspects of the disease including degeneration of dopaminergic cells and formation of Lewy body-like inclusions (*8, 9*). These flies also show age-dependent loss of movement control. The *Drosophila* model of Parkinson's disease can also be treated by some of the same drugs including dopamine agonists with positive results (*10, 11*). Although flies have no homologues to *α-synuclein*, they do have their versions of another two genes that genetically cause Parkinson's disease—*parkin* and *pink1*. In flies, *parkin* appears to be downstream of *pink1* in the same pathway (*12, 13*). Mutations in these genes lead to mitochondrial defects, muscle and locomotor dysfunction, but do not damage dopaminergic neurons (*14, 15*). On the other hand, overexpression of *parkin* in flies can suppress the effect of human α-synuclein-dependent degeneration phenotype (*16*). Co-overexpression of HSP70 and α-synuclein in the fly brain can rescue dopaminergic neurons against α-synuclein-induced neurodegerative phenotype (*17*), revealing potential therapeutic targets.

Alzheimer's disease has also been modeled in flies. Unlike the Parkinson's disease model, there are homologues in *Drosophila* to the human Alzheimer's disease-associated genes—the *amyloid precursor protein* (*APP*) gene and *Presenilin-1*. Just as in humans, Presenilin (fly version) is responsible for the release of the A*β* peptide from APP via proteolytic cleavage. The hallmark lesion in Alzheimer's disease is characterized by the formation of A*β* peptide-containing amyloid plaques in brain [reviewed in (*18, 19*)]. The mechanism of APP processing has been investigated in *Drosophila* using a genetic screening approach that showed *Drosophila* Presenilin is involved in the cleavage of the Notch protein (*20*). *Drosophila* APP appears to participate in axonal transport and if misexpressed leads to axonal vesicular accumulation (*21–23*). Fly models of Alzheimer's disease have striking similarities to phenotypic defects resembling Alzheimer's disease, in particular age-dependent learning defects, progressive neurodegeneration, and protein aggregate formation (*24–28*).

Even more impressive is the finding that the ubiquilin protein (UBQLN1), which when mutated can cause AD, can suppress Psn overexpression-induced phenotypes in flies (*29*).

The second hallmark of Alzheimer's disease, tauopathy, has also been modeled in flies. tau is a microtubule-associated protein and the principal component of the neurofibrillary lesions that can be associated with some amyloid plaques. Currently, *tau* receives more attention in the emerging view that the synergistic action of Aβ and tau is causal in Alzheimer's disease (*30*, *31*). *Drosophila* models have been particularly instrumental in understanding the relations between tau and the Aβ peptide-containing amyloid plaques. When wild type and mutant forms of human tau are expressed in *Drosophila*, flies recapitulate the major human disease phenotypes, including progressive neurodegeneration, accumulation of abnormal tau, and neurotoxicity. The Alzheimer's disease-like neurofibrillary pathology is also observed when expression of wild-type human tau is combined with its *Drosophila* GSK-3 homologue (*32*), suggesting that GSK-3 may be a potential drug target. Expression of wild-type human tau also causes impaired axonal transport with vesicle aggregation and loss of locomotor function (*33*). *Drosophila* models support a role for cell-cycle activation, leading to apoptosis of postmitotic neurons *in vivo*. As in Alzheimer's disease, target of rapamycin kinase (TOR) activity is increased in fly models and promotes neurodegeneration. TOR activation enhances tau-induced neurodegeneration in a cell cycle-dependent manner and, when ectopically activated, drives cell-cycle activation and apoptosis in postmitotic neurons (*34*).

Possibly the most successful area of human neurological disease modeling in *Drosophila* is the models of polyglutamine tract repeat disorders [reviewed in (*35*)]. The fly eye is an excellent readout for polyglutamine tract repeat disorders like Huntington's disease and the spinocerebellar ataxias. In both conditions, there is a critical threshold of polyglutamine repeats that must be reached before a clinical presentation is observed. In flies, expression of the human Huntingtin protein or the SCA3/MJD protein containing the clinically relevant number of repeats leads to degeneration of photoreceptor neurons (*36*, *37*). As in humans, these defects become more severe as the flies age and can become quite extreme as the number of polyglutamines increases suggesting conserved mechanisms. Using the *Drosophila* eye for misexpression studies provides an easy and convenient opportunity for genetic screening approaches. In screens for mutations that modify polyglutamine repeat phenotypes, it has been found that heat-shock proteins HSP70, HSP40, and other proteins can ameliorate these defects and may even serve as neuroprotectors (*38–40*). In fact, there may even be shared pathways between the SCAs and HD which suggest that these fly models could produce therapeutic targets that will work for all polyglutamine expansion disorders (*41*). There is even hope that polygluatime repeat disorder fly models could be used to screen for small molecules that can suppress these phenotypes as well.

III. *Drosophila* in Cancer Research

The major functional components of the cell cycle and metabolic and signaling pathways leading to cancer are highly conserved between fruit flies and humans. *Drosophila* is quite useful in modeling cancer or at least simple morphological aspects of cancer such as cell division, apoptosis, or cell migration [reviewed in (*42–47*)]. It should also be noted that the fruit fly has a glorious past serving in studies on the delineation of signaling pathways involved in oncogenesis. For example, signaling by Wnt proteins (Wingless in *Drosophila*), Ras/MAPK, Notch, and Hedgehog have well-characterized roles during the fly's embryonic development and in adults. All of these signaling pathways are clearly implicated in mammalian tumorigenesis and metastasis [reviewed in (*48–52*)].

Modeling human disease in flies, especially cancer, may at first seem a bit overambitious, except for the fact that at the molecular level, these cell-cycle genes and the process of cell proliferation, cell division, and cell motility are highly similar among multicellular organisms. Therefore, the fly becomes a genetic model for the identification of pathway members and not a model that recapitulates the cell biological characteristics of cancer such as tumor growth, differentiation, and vascularization. For example, the mosaic technique in flies provides the ability to work with strong or lethal mutations during various stages of development or in the adult tissues. Animals homozygous for a strong cell cycle or growth mutation could be lethal at very early stages of development without obvious morphological defects making them hard to study. The mosaic technique makes it possible to generate homozygous clones of lethal mutations in an animal that is otherwise heterozygous (i.e., morphologically wild type) for the same mutation [reviewed in (*53*)]. Genetic mosaicism of these chimeric flies mimics the loss of heterozygosity observed in the somatic cells of cancer patients. Mosaic flies carrying mutations can display clones of cells with irregular growth and overproliferating phenotype. This technique allows for the examination of homozygous mutant phenotypes and the design of genetic screens to identify tumor suppressor genes.

Using this mosaic analysis method in a nonvital somatic tissue like the compound eye led to the identification of the tumor suppressor gene *archipelago* (*ago*). This screen was designed so that mutants that display increased cell proliferation could be easily identified using clonal analysis in the *Drosophila* compound eye (*54*). The F-box protein Archipelago is involved in a mechanism that suppresses cell proliferation by promoting the degradation of Cyclin E, a protein required for entry into S phase, the DNA synthesis phase of the cell cycle (*54*). Its human orthologue, *hCDC4/hAGO* has a similar function and, perhaps not surprisingly, is mutated in some breast and ovarian cancer cell lines (*54–56*). In addition, up to 16% of endometrial carcinomas may be the result of *hCDC4/hAGO* mutations (*57*). Mutations in another gene identified in this

screen, *erupted*, the *Drosophila* orthologue of mammalian *tumor susceptibility gene 101*, causes dramatic non-cell-autonomous overproliferation of adjacent wild-type tissue (*58*). In a different screen for *Drosophila* mutations that result in tissue overgrowth, *salvador* (*sav*), a gene that promotes both cell cycle exit and cell death, was identified. The human orthologue of *salvador* (*hWW45*) is also mutated in cancer cell lines (*59*).

In addition to cell overproliferation phenotypes, *Drosophila* models have been used to study developmental signaling pathways that regulate pattern formation and cell migration. In particular are the processes that model cell motility, a critical step in tumorigenesis and metastasis (*60*, *61*). During normal development and also during tumorigenesis, cells change their position extensively. The basic mechanisms involved in cell locomotion have been studied primarily *ex vivo* in cultured cells. An obvious disadvantage of this approach is that these cultured cells are now isolated from the comprehensive signaling networks that underlay guided cell migration *in vivo*, not to mention tissue-specific cellular interactions. Recently, major advances have been made in the study of migrating cells in *Drosophila* and have shed light on the basic mechanisms of cell locomotion. These studies of cell migration take place in a number of cell types including hemocytes (embryonic blood cells), primordial germ cells, border cells in the ovary, and tracheal cells.

A number of elegant studies in *Drosophila* using a variety of genetic approaches have contributed to our current understanding of guided cell migration. *Drosophila* genetic screens have uncovered new genes that are relevant to human cell migration, including tumor invasion and metastasis [reviewed in (*44*, *62–65*)]. For example, loss of function genetic screening in mosaic clones revealed that Taiman, the p160-type steroid hormone coactivator, is required for the border-cell migration and the proper distribution of adhesion molecules (*66*). The human homologue of *Taiman*, called *Amplified in Breast Cancer 1*, is upregulated in many ovarian and breast cancers (*67*). Taiman acts as a coactivator for the estrogen receptor. Blocking estrogen signaling in cancer patients can prevent metastasis and recurrence (*68*). Understanding more about function of Taiman may provide insight into its role in estrogen receptor signaling and mechanisms of metastasis. On the other hand, Taiman has been reported to have a role in cell migration independent of its role in estrogen receptor signaling. SRC-3, a homologue of Taiman, promotes cell migration of human ovarian cancer cells regardless of the estrogen receptor status of the cells (*69*).

Border cells in the *Drosophila* ovary are a group of 6–10 epithelial cells that become invasive and eventually migrate to the oocyte border. Studies of border-cell migration have revealed that transformation of nonmotile cells within follicular epithelium into invasive cells occurs via activation of the JAK/STAT signaling pathway through the Domeless receptor (Dome) (*70–72*). Similar JAK/STAT-dependent signaling mechanism is also applied in tracheal

cell migration in response to activated expression of Trachealess and the FGF receptor (*73*, *74*). Cancer cells appear to use similar mechanisms: some mammalian STATs are upregulated or activated in cancer cells, in fact, STAT3 can promote cell-cycle progression and protect against apoptosis (*75*).

An interesting connection between cell motility and programmed cell death was recently revealed in *Drosophila*. DIAP1, the *Drosophila* inhibitor of apoptosis protein (IAP), is required for border-cell migration (*76*). IAPs are evolutionarily conserved proteins that bind to caspase proteases blocking their activity and thereby inhibiting apoptosis. IAPs also control cell growth during carcinogenesis [reviewed in (*77*, *78*)]. One human IAP, XIAP, is a key determinant of sensitivity to cisplatin in ovarian cancer cells, and failure of cisplatin to downregulate XIAP is a hallmark of chemoresistance (*79*, *80*). In border cells, DIAP1 was identified in a genetic overexpression screen to rescue the migration defect caused by expression of a dominant-negative form of the small GTPase RAC1 (*76*). Although the mechanistic explanation of these phenomena has yet to be uncovered, given the functional similarities of border cells and ovarian cancer cells, it seems reasonable to predict that XIAP or another human IAP could contribute to cell motility in ovarian cancer cells.

Useful cancer-suppressing therapeutic agents may also be developed as a result of studies in *Drosophila*. Genetic screening in the Hedgehog pathway first clarified that to initiate a signaling cascade that regulates early tissue differentiation, the *hedgehog* gene interacts with the transmembrane protein *patched* and its partner *smoothened*. Cyclopamine, a compound in the corn lily, *Veratrum californicum*, was found to act as an inhibitor of *smoothened* and, as a consequence, suppressor of *hedgehog* signaling (*81*, *82*). Mutations in the human homologue of *patched* have been reported in basal cell nevus syndrome (OMIM #109400), also known as Gorlin syndrome. *Drosophila* studies suggest the possibility that topical cyclopamine could be potentially beneficial in the treatment of skin cancer in humans (*83*, *84*).

IV. Tumorigenesis, Neuroprotection, and Fortitude: The Hypoxic Response in *Drosophila*

Oxygen deprivation, or hypoxia, and the cellular mechanisms that can regulate hypoxia are key factors in the pathogenesis of cancer, stroke, and familial inherited disorders such as those that occur in Von Hippel-Lindau syndrome (OMIM #193300) [reviewed in (*85–87*)]. For example, localized hypoxic effects play a central role in limiting tumor growth and may also be involved in blunting the actions of important chemotherapies [reviewed in (*88*, *89*)]. Oxygen deprivation causes devastating effects during acute ischemic injury and the

accompanying cardiac infarct and stroke often lead to brain injury. In fact, some neurons in the brain are particularly vulnerable to hypoxia causing rapid irreversible damage. While complete interruption of blood flow can kill cardiac myocytes or kidney cells in 20–40 min, it takes less than 5 min to start trigger the death of neurons in brain [reviewed in (*90*, *91*)]. Using simple genetic model organisms like *Drosophila* to delineate the mechanisms of hypoxic response and adaptations to low oxygenation these animals have acquired may provide new tools in therapeutic interventions to preserve the status quo in hypoxia-vulnerable cells, increase their tolerance for lower oxygen, and promote survival.

Many nonmammalian organisms can tolerate extended hypoxic episodes (*92*, *93*). Studies in yeast and in zebrafish embryos had demonstrated that development in these organisms can be arrested reversibly in response to hypoxia (*92–95*). The ability to temporary shut down metabolism is not limited to embryos or early stages of development. Cold-blooded animals like turtles are known to hibernate in wintertime in essentially complete anoxia (*96*). Even some small mammals like mice can survive several hours of hypoxia with little or no neurological damage. Animation state in mice can be suspended by H_2S-induced strong suppression of oxygen use (*97*). In these conditions, the body temperature is dropping but the mouse can survive hypoxia for a few hours (*98*).

It is not surprising that *Drosophila* also exhibits a protective response to hypoxia because flies spend their embryonic and larval life stages submerged in rotting fruit, where they must compete for limited oxygen supplies. Early work on oxygen deprivation in flies showed that the initial mitotic cycles of fly embryos can be temporary arrested by hypoxia and embryos remain viable despite prolonged periods of hypoxia (*99*, *100*). As one might expect, *Drosophila* tolerates much longer exposures to hypoxic conditions than mammals (*101–104*). In fact, even after a week in the near absence of oxygen, arrested embryos recover and develop when oxygen is restored.

Recent studies have revealed a sophisticated signal transduction system that has evolved to ensure animals survival during hypoxia. Activation of this signaling system alters the behavior of the organism and stalls cell proliferation. Although little is known about the mechanisms that elicit the rapid metabolic turnover upon sudden reduction of oxygen supply, some evidence suggests the contribution of processes that trigger a rapid switch to energy conservation upon abrupt imposing of severe hypoxia [reviewed in (*105*, *106*)]. The powerful genetic manipulations available in *Drosophila* may prove to be the key tools required to answer the profoundly difficult question of identifying ways to enhance the survival of hypoxia-sensitive cells.

Considerable progress has been made in understanding the aspects of hypoxic response under control of a transcription factor called the hypoxia-inducible factor 1 (HIF-1). HIF-1 plays a pivotal role in cellular adaptation to oxygen availability and is directly regulated by ubiquitin-dependent machinery

[reviewed in (*107*)]. HIF-1 is composed of two subunits, the oxygen-sensitive HIF-1α and constitutively expressed HIF-1β. When oxygen levels are normal, the HIF-1α subunit of this factor is targeted for ubiquitination and subsequent rapid degradation by the ubiquitin proteasome system (*108, 109*). This process involves the modification of HIF-1α by prolyl hydroxylases, or PHDs (*108–111*). Hydroxylated HIF-1α is then recognized by the E3 ubiquitin ligase that contains the product of the *von Hippel-Lindau* (or *VHL*) gene, a tumor-suppressor gene, and targets it for degradation (*112–114*). Hypoxic conditions stabilize the HIF-1α protein and allow it to accumulate in the cell. HIF-1-induced transcription contributes to hypoxia response that allows cells to accommodate to at least mild reductions of oxygen (*112–116*).

A homologous hypoxia-responsive system has been described in flies. *Drosophila* bHLH-PAS proteins Similar (Sima) and Tango (Tgo) are HIF-1α and -β orthologues, respectively (*108, 117, 118*). *Drosophila* orthologues of VHL, or dVHL, and of PHD, encoded by the *hph* gene, have also been identified (*118–121*). Like in mammals, *Drosophila HIF-1β* homologue *tango* is constitutively expressed regardless of oxygen conditions, while *HIF-1α* homologue *sima* is rapidly degraded in normoxia and stabilized in hypoxia (*108, 117, 118*). Following its mammalian homologue pattern, normoxic Sima degradation in flies depends on the activity of a conserved prolyl-4-hydroxylase, *Drosophila* PHD (*118*).

The HIF-dependent transcription is peaking when oxygen is lowered to 5% (vs normal 20.8%). Flies can generally adapt to mild hypoxic conditions and even continue to grow and reproduce (*122*). However, under severe hypoxic conditions (<1% oxygen), they struggle to survive. At 1% oxygen or below, *Drosophila* embryos enter a state of suspended activity, whereas larvae will attempt to escape from the oxygen-poor environment (*123*). The responses to mild hypoxia are under the control of HIF (*124*), whereas the response to severe hypoxia involves distinct controls that are largely independent of gene expression (*125*). Abrupt termination of oxygen supply immediately arrests nearly every metabolic process in flies: cell cycle, cell motility, gene expression, turnover of nucleic acids, and proteins. Embryos typically retain about 75% of their ATP, indicating that they are conserving limited reserves (*126*). Once oxygen is restored, even after several days, embryos with suspended activity resume their development and normal flies can be produced.

Drosophila larvae, deprived of oxygen, exhibit behavioral changes that are related to a larval phenotype governed by a protein kinase G (PKG) allele (*127*). PKG is involved in one pathway with nitric oxide (NO), which is a well-known regulator of hemodynamics in humans, suggesting that cellular, developmental, and behavioral responses to NO mimicked those induced by hypoxia. Genetic and pharmacological tests showed that NO mediates at least some of these responses (*123*).

Powerful unbiased genetic screening methods available in *Drosophila* have been applied to identify hypoxia-sensitive mutants. Genes *hypnos-1*, *hypnos*-2, and *hypnos-3* were isolated in a screen for mutants with slow recovering mobility after a 5-min period of hypoxia (*102*). *hypnos*-2, a pre-mRNA *adenosine deaminase* and *dMRP4*, a homologue of a human *multidrug resistance protein* (*128*, *129*) were also identified in this screen. The *hypnos*-2 mutants were implicated in RNA editing, in neuronal function, and in the response to hypoxia (*129*). Knocking down HIF-1α partially restores sensitivity to chemotherapy including levels of adriamycin, etoposide, and others and may be due to the regulation of multidrug resistance proteins by HIF-1α.

Drosophila models may be of particular interest with regards to nutrition-dependent mechanisms of hypoxia tolerance. In flies, the response to chronic hypoxia appears to be not only strongly dependent on diet but is also largely age independent (*130*, *131*). Remarkably, a rich diet (more sugars and proteins) promotes sensitivity to chronic hypoxia in *Drosophila*, whereas starvation increases the life span in hypoxia conditions (*130*, *131*). Although difficult to directly compare to humans, some studies suggest that calorie restriction decreases cancer risk in mammals (*132*, *133*).

V. Blood, Immune Response, and Infectious Disease

Innate immunity is a phylogenetically ancient protection mechanism. It serves as the first line of defense against infection by foreign pathogens. Evolutionary conservation of biochemical pathways involved in innate immunity make *Drosophila* a powerful model to study the prototypical immune response.

Flies, just like humans, also suffer from infectious disease. A fly could not survive without mechanisms to constantly defend itself against pathogens in its native environment. Despite the fact that immune response in flies, like in all invertebrates, does not involve a T-cell response or the production of specific antibodies against foreign proteins, there are many similarities in innate immunity between flies and humans. Many striking parallels can be drawn between functions of circulating cells, like leukocytes, as well as transcription factors and signaling pathways, such as GATA factors, JAK/STAT, or Notch pathways that regulate hematopoesis and immune response [reviewed in (*134*, *135*)].

Mammalian blood contains three distinct groups of cells: red cells, white cells, and platelet. Red blood cells deliver oxygen, the platelet group promotes clotting, while the white blood cells provide immunity and scavenge dying cells. Mammalian hematopoiesis occurs in two waves called primitive and definitive hematopoiesis, equivalent to *Drosophila* embryonic and larval hematopoiesis, respectively. *Drosophila* hemolymph performs all blood-like circulation duties except delivering oxygen.

There are three lineages of *Drosophila* blood cells, or hemocytes: plasmatocytes, crystal cells, and lamellocytes. The plasmatocytes are the predominant *Drosophila* blood cell line and equivalent of the cells from the mammalian monocyte/macrophage lineage. They play a critical role in the phagocytosis of invading microorganisms, engulfment of apoptotic cells, and tissue remodeling (*134*). Other immune functions including melanization and encapsulation are performed by the less frequently distributed crystal cells and lamellocytes in *Drosophila*.

Although fly hematopoiesis is simpler compared with mammalian hematopoiesis, many processes responsible for making blood cells are well preserved throughout evolution. Blood cell development in humans and flies is regulated by several remarkably homologous transcription factors and signaling cascades. *serpent*, a transcription factor and *Drosophila GATA* orthologue is required for the hemocyte development in flies (*136, 137*). The *Friend-of-GATA* homologue *U-shaped* has been found to block crystal-cell development (*138*). The family of Friend-of-GATA multiple zinc-finger proteins is known to regulate GATA activity in mammals [reviewed in (*139*)]. In mice, *Friend-of-Gata-1* is involved in regulation of erythropoiesis and megakaryopoiesis (*140*). In flies and vertebrates, both GATA factors and Friend-of-GATA are under the control of BMP signaling. Dpp, similar to its vertebrate counterpart BMP2/4, regulates *srp* and *ush* transcription (*141*).

lozenge, another transcription factor involved in crystal cell formation (*142, 143*), contains a "RUNT" domain homologous to a human transcription factor *AML1/RUNX1. AML1* was originally isolated as a fusion partner in a chromosomal translocation associated with acute myelogenous leukemia and found to be necessary for definitive hematopoiesis [reviewed by (*144*)]. The expression of *lozenge* in larvae appears to be under the control of the Notch pathway (*145*). Notch signaling has been widely implicated in the regulation of hematopoiesis in mammals [reviewed in (*146, 147*)].

The *Drosophila* orthologue of the vertebrate gene encoding early B-cell factor-1, the transcription factor named *collier*, is required for lamellocyte specification (*148*). *Drosophila* larvae mutant for *collier* fail to produce lamellocytes on parasitization (*148*).

The Janus kinase signal transducers and activators of transcription (JAK-STAT) have been implicated in conserved regulation of blood cell development. Hyperactivation of Hopscotch, the *Drosophila* JAK homologue, causes hemocyte overproliferation and melanized tumor formation (*149–151*). In humans, hyperactivation of STAT homologues is associated with various leukemias and lymphomas (*152, 153*).

Normal plasmatocytes development and their migration to the posterior end of the embryo are regulated by receptor tyrosine kinase pathway that requires the activity of the PDGF/VEGF receptor, or PVR (*154*). PVR is a