

Pulmonary Perspective

Drosophila in Asthma Research

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Genetic research has revealed a number of asthma-susceptibility genes. In addition, with the development of genome-wide association studies, which has gained unprecedented momentum, the roles of many more candidate genes in asthma will be uncovered. In parallel with such genetic insight, a detailed understanding of the function of susceptibility genes in asthma is required, a task best suited for genetically tractable model organisms. The inherent limitations of models like the mouse necessitate finding complementary systems for study. Although the fruit fly *Drosophila* has not been used previously in asthma-related research, it might prove to be extremely helpful in relating genetic processes to biological function. We discuss the usefulness of the *Drosophila* model by analyzing potential homologs of known asthma-susceptibility genes in the fly. Except for those associated with adaptive immunity, we and others found unequivocal orthologs for all of them. Most asthma-related genes are indeed expressed in the airway epithelium. In addition, some are regulated upon airway infection of the *Drosophila* airway epithelium, pointing to an important role in airway immunity and development of asthma-like phenotypes in the fruit fly. Finally, high throughput functional analyses are needed to complete genome-wide comparison studies in complex diseases such as asthma. Because such studies are most readily performed in the fruit fly, it may be a particularly useful asthma model system.

Keywords: *Drosophila*; genome-wide association studies; susceptibility genes; animal model

Asthma is the most common chronic inflammatory disease of the lung. Like most inflammatory diseases of barrier epithelia, asthma is a typical complex disease, meaning that different intrinsic and extrinsic factors contribute to disease development. Asthma has a definite genetic component and heritability accounts for 50 to 60% of cases. Family and twin studies revealed that the risk for developing asthma is increased fivefold in first-degree relatives of patients with asthma (1). Positional cloning and candidate gene studies have provided researchers with a large number of asthma-susceptibility genes. Their identification has suggested that new and unexpected pathways are involved in asthma development, increasing the number of potential targets for pharmacological intervention (2, 3).

New generations of genome-wide association studies (4) performed with large and well-defined cohorts of patients will provide us with an almost complete set of asthma-susceptibility genes in the near future. Although this body of information is of central importance, its usefulness is currently limited by our

inability to understand the connection between most of these genes and disease development. Thus, asthma research faces a situation common to that experienced for all complex diseases: the identification of susceptibility genes is the beginning rather than the end of the endeavor (2). Studies in suitable model organisms are therefore of paramount importance for elucidating the physiological and pathophysiological relevance of the genes and genetic variations identified. Transgenic mice, including “humanized” mice, are currently the only genetically tractable models used in asthma research (5). Although they have been and will be extremely useful, transgenic and mutant mice have drawbacks that obscure a direct analysis of the expected phenotypes. Genetic redundancy and developmental plasticity are two general problems associated with transgenic or mutant mouse studies. In addition, it is technically not a trivial task to produce transgenic or mutant mice tailored to the needs of lung physiologists. To supplement this technical resource, other animal models, especially those with less complexity, are needed. Alternative prototype organisms will be required to more readily address the recent discoveries that innate immunity and airway epithelial cells are major determinants of asthma development. The fruit fly *Drosophila melanogaster* is outstanding among these invertebrate model organisms because it combines a comparatively simple physiology and genetic organization with an unparalleled tool kit for genetic manipulation while possessing the major organs relevant to asthma.

DROSOPHILA AS A MODEL IN ASTHMA RESEARCH?

Drosophila has not previously been used as a model for asthma research. Apparently, this stems from the fact that flies are devoid of an adaptive immune system, meaning that they do not possess the hallmarks of allergic diseases such as immunoglobulins, T cells, and most aspects of interleukin signaling (6, 7). Nevertheless, new developments in asthma and fly research may transform limitations of the *Drosophila* model into considerable advantages.

More asthma-susceptibility genes are involved in innate immune responses or in maintaining epithelial cell integrity than in processes associated with adaptive immunity (3). The central position of epithelial cells for disease development has been emphasized in recent physiological studies. Innate immune signaling systems within epithelial cells are necessary and sufficient for experimental disease induction (8, 9). The identification of these two major determinants of asthma development, the airway epithelial cell and the innate immune system, makes the fly attractive insofar as it is well suited for studying the roles of these two aspects. Flies depend solely on their innate immune system because they do not possess adaptive immunity, which allows an exclusive view of the role of innate immunity under different physiological conditions. The airways of the fly (tracheae) have a simple architecture made of interconnected tubes terminating in a multitude of fine blind ending structures (Figure 1A). In its hierarchical organization with primary, secondary, tertiary, and terminal branches, the fly airway resembles the overall design of

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the human lung (10, 11). The entire organ is made of epithelial cells only, forming a single cell layer around the central airspace. Although of low complexity, it is a highly effective structure and shares numerous physiological similarities with our own lung (12). This unique and simple structure can be seen and treated as a cell culture of airway epithelial cells in a normal animal. The epithelial cells of the fly trachea, regardless of their localization within this organ, are able to launch an immune response if confronted with pathogens (13). Although epithelial cells from *Drosophila* and humans share substantial similarities, differences exist, such as the absence in the fly of both ciliary transport and the oxygen transport function throughout its entire airway system. Another dissimilarity is the inability of the fly's airway epithelium to recruit blood cells even after prolonged activation. This distinction allows for an unobstructed analysis of the role of epithelial cell innate immunity.

Drosophila offers intriguing experimental advantages that are not shared by other model organisms. Numerous deletions (14) and more than 12,000 transposable element insertions are readily available to researchers for creating genomic mutations (15). Numerous vectors for transgenesis are also available (16) and some have been developed that capitalize on *P* element, ϕ C31, and recombineering technologies (17). One highly useful tool is the binary GAL4/UAS-system invented more than a decade ago (18) that relies on the yeast transcription factor GAL4 and the corresponding DNA binding region, UAS. This system conveniently allows overexpression or silencing of specific genes in the organ of interest. These two components are segregated into different fly lines, namely, the overexpression or silencing construct of the gene under transcriptional control of UAS elements, and the GAL4 line that determines the site of expression (19, 20) (Figures 1B and 1C). Crossing these two lines activates this system in the entire F1 generation (Figure 1D). As the parental strains are not impaired, researchers can induce silencing of various genes of interest in one organ (21) or overexpression of another gene in a different set of organs simply by crossing the corresponding lines (15). Especially relevant to asthma research is the airway epithelium, but also hematopoietic cells and the brain might be of interest, all of which can be targeted specifically

by publicly available driver lines. For the airway epithelium, some drivers are available that direct expression into the entire set of airway epithelial cells or into only well-defined subsets of them (Figures 1E and 1F), which permit expression or RNAi-mediated gene silencing.

Further temporal control of expression can be achieved using the tripartite temporal and regional gene expression targeting (TARGET) system (22). In this system, a temperature-sensitive repressor of GAL4, GAL80ts, represses expression under restrictive conditions (19°C). Shifting to the permissive temperature (29°C) inactivates the temperature-sensitive repressor, thus releasing GAL4 from its inhibition, which leads to expression of the target gene. Because this process is reversible, returning to the restrictive temperature can terminate expression. Such a TARGET expression system has been established for the most specific airway epithelial driver, *ppk4*-GAL4. This versatile and simple system enables the researcher to exclude developmental effects of overexpression or silencing and to separate transient from chronic effects simply by choosing an appropriate activation regime.

Recently, Barry Dickson's group completed the great endeavor of producing RNAi lines for all annotated *Drosophila* genes and provided this collection to the *Drosophila* community (23). The combination of these publicly available lines with the GAL/UAS system allows efficient gene silencing in most parts of the organism, or in an organ-specific fashion, depending on the GAL4 driver used. In addition, "humanized" flies carrying transgenic arrays of the corresponding human genes, or genetic variations of them, have been successfully used, especially for studying the molecular and cellular basis of neurodegenerative diseases (24). Production of these fly strains has potential in various fields, including asthma and related diseases of the airways, and is simpler and less time consuming than making sophisticated transgenic mice. As mentioned previously, comprehensive studies are possible with a small number of stocks due to the binary nature of the GAL/UAS system that allows free combination of driver and effector lines (19). The GAL4/UAS system exemplifies only one of the many tools available to *Drosophila* researchers. Other extremely powerful techniques exist that maximize the abilities of what can be studied in the

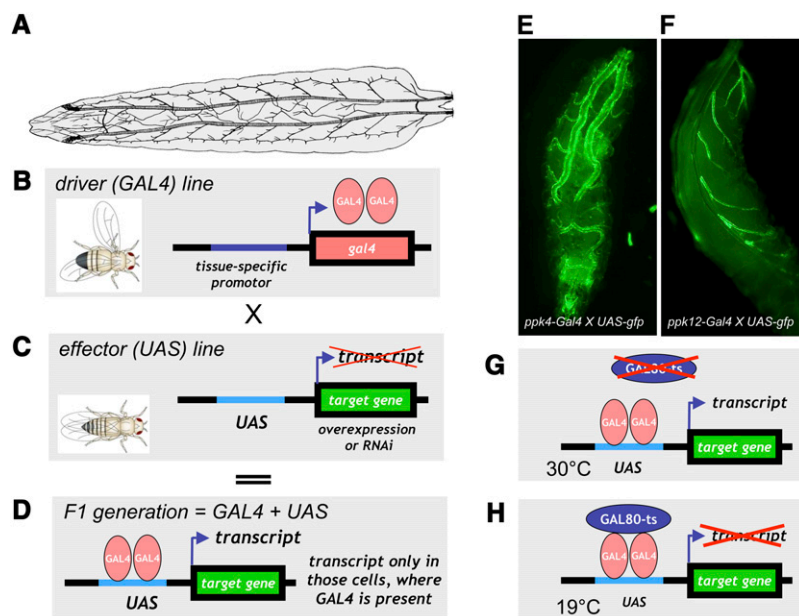


Figure 1. (A) A series of interconnected tubes constitutes the airway system (trachea) of the larval and adult fly. It is made exclusively of an epithelial cell layer around a central airway. The tubes are hierarchically organized in primary, secondary and terminal structures. The larval tracheal system is shown. (B–F) The GAL4/UAS-system is ideally suited to target expression of genes of interest or of RNAi constructs to the organ of interest. It is a binary system consisting of a GAL4 or driver line that determines (B) the site of expression and (C) the UAS or effector line that carries the gene to be expressed under transcriptional control of the UAS element. Both elements of the binary system are segregated into two different fly lines. (D) Using simple crossing, both elements are combined, leading to expression of the genes of interest in the organ of interest. For *Drosophila* a number of different driver lines are available that direct expression of the effector gene (in this case GFP) to (E) the entire airway epithelium or (F) only a subset of epithelial cells. (G, H) A refinement of the binary GAL/UAS-system is the TARGET system that allows additional temporal control of expression. It adds a temperature-sensitive repressor of GAL, called GAL80ts, to the system. At repressive temperatures (19°C), (G) the entire system is inhibited, whereas at permissive temperatures (29°C), (H) the temperature sensitive repressor is inhibited thus releasing GAL4 from its inhibition.

fly, and it is impossible to summarize all of them here. The reader is referred to additional reviews for more detailed discussions of *Drosophila* genetics (25, 26).

To evaluate if *Drosophila*, with all its advantages, can develop into a suitable model for asthma research, we focused on the canonical set of asthma-susceptibility genes (2, 3) and tried to find homologs in the fly. A smaller group of these genes, associated with adaptive immunity, have no counterparts in the fly. These comprise genes such as IL-4, IL-10, IL-13, IL-18, IL-4R, CC16, CTLA4, HLA-DBP1, HLA-DQB1, HLA-DRB1, LTA, LTC and FCER1B. For all other candidates, we found orthologs in the fly genome. This analysis is summarized in Table 1. To evaluate if they may be useful, we checked if these genes are expressed in the airway epithelium. Expression data for most organs can be extracted from an online depository (27), or, in the case of the airway epithelium, from our own studies (13) (C. Wagner, personal communication). As listed in Table 1, most of these genes are indeed expressed in the airways, indicating that their study might be useful to elucidate their role in normal airway physiology but also in pathological situations. In addition, some of these candidates are regulated upon infection or ectopic activation of the epithelial immune system within the airway epithelial cells. For most of these *Drosophila* orthologs, informative genetic material is available comprising conventional knock out strains, those that allow tissue-specific gene silencing, and additionally in some cases those that allow tissue-specific overexpression (Table 1).

The airway epithelium of the fly is accessible for two major types of experimental manipulation with relevance for asthma. In one technique, infection of the airways with different bacteria can be achieved using a simple experimental protocol where larvae are confronted for a brief period with high concentrations of bacteria. Because this is performed at slightly reduced temperatures, oral uptake is greatly reduced, thus shifting the infection process toward tracheal uptake (28). Using transgenic indicator lines that carry a *P* element with GFP under transcriptional

control of an antimicrobial peptide gene promoter, the innate immune response of the airway epithelium can easily be visualized (28). With another paradigm, the immune response of the airway epithelium can be activated by ectopic expression of the pattern-recognition receptor PGRP-LE using the GAL4/UAS system (28, 29). Driving expression with particularly strong GAL4 lines such as this induces modifications of the airway epithelium that resemble those found in asthma, including metaplasia of the epithelium and obvious problems with oxygen delivery (C. Wagner, personal communication). This asthma-related phenomenon can also be observed after strong immune reactions of the epithelium (28).

After implementing either procedure, identification of disturbances within the tracheal system can be monitored using two very simple but nevertheless informative approaches. The transparency of the larvae allows a visual evaluation of the tracheal structure such that functional impairments often caused by liquid-filled parts of the tracheal lumen are easily identified. A physiological test of the operability of the tracheal system takes advantage of the normal behavior of the larvae. Healthy animals are usually buried in their food, but upon oxygen deprivation they move to the surface, which is also observed in larvae that show impairments of the tracheal system. This behavior can be quantified (time to leave food or percentage of larvae showing this behavior), thus giving a measure that provides information about the supply of oxygen to tissue.

Besides the comprehensive collection of methods and tools available for *Drosophila*, one major advantage is its relatively simple genomic organization. Usually, gene families that have numerous members in mammals are composed of fewer or sometimes only one member in the fly, which is exemplified by the matrix metalloproteinases (MMPs). Humans have 23 MMPs with different but partially overlapping physiological roles, whereas *Drosophila* has only two MMPs (30). Because the gene MMP9 is considered an asthma-susceptibility gene (Table 1), its *Drosophila* homolog (DmMMP1) could be

TABLE 1. HOMOLOGS OF ASTHMA-SUSCEPTIBILITY GENES IN THE FRUIT FLY *DROSOPHILA*: THEIR EXPRESSION, RELEVANCE FOR AIRWAY PHYSIOLOGY AND THE AVAILABILITY OF GENOMIC RESOURCES

Asthma Gene	<i>Drosophila</i> Homolog	Expressed in Airways	Regulated or Relevant in Airways	Genomic Resources
GSTM1	GST-D family	+	D1, D2, D3, D9	all: KO, RNAi
GSTT1	GST-E family	+	E1, E5, E7	all: KO, RNAi
GSTP1	GST-S1	?		KO, RNAi
ADAM33	Neu3(CG7649), mmd(CG42252)	?, ?		all: KO, RNAi
MMP9	DmMMP1	+	DmMMP1	KO, RNAi, OE
ORMDL3	dORMDL	+		RNAi
FLG	CG1433 Atu	?		KO, RNAi
STAT6	STAT92	+	STAT92	KO, RNAi, OE
ADRB2	Oct2β-like	+	Oct2β-like	KO, RNAi
ACE	ANCE-family	+	ance-2	RNAi
NOS	dNOS	+		KO, RNAi, OE
DPP10	ome, CG17684	?, +		both: KO, RNAi
NAT2	aaNAT-1	?		KO, RNAi
SPINK5	Serpins	+	spn77Ba	KO, RNAi
TNF	Eiger	?		RNAi
GPRA	CcapR, AICR2, TakR86C	?, +, +	AICR2	all: KO, RNAi
CMA1	trypsin29F, snk	?, +		RNAi; KO, RNAi
CCL11, CCL5	upd1-3	+, +, ?	upd-2	KO, RNAi
TBXA2R	CG7497, OAMB	?, +		KO, RNAi; KO, RNAi, OE
TGFβ1	activin, maverick, gbp	?, ?, ?		RNAi, -; RNAi
CD14	CG5195	?		RNAi
TLR2, 4, 6, 10	Toll receptors	+	Toll, 18-w, Toll-7, Toll-8	all: KO, RNAi

Listed are the homologs of canonical asthma-susceptibility genes. Expression data are from unpublished work of our group and refer to transcription data. The genomic resources available have been extracted from the Vienna *Drosophila* RNAi Center (VDRC) (17), Bloomington *Drosophila* Stock Center (BDSC) and *Drosophila* Genomics Resource Center (DGR) (see FlyBase, www.flybase.org). Genomic KOs refer to null/hypomorphic alleles or to lines carrying *P*-elements in the corresponding genes allowing easy production of genomic deletions. OE = GAL4/UAS mediated overexpression lines; + = transcripts were identified; - = this resource is not available; ? = no information; all = information valid for all homologs listed in the fourth row; both = the situation where only two homologs are listed.

analyzed to determine its function independently of the other 22 MMPs.

Drosophila appears to be a promising model for the study of Toll receptors in the role of asthma. Some Toll-like receptors such as TLR2, 4, 6, and 10 are asthma-susceptibility genes (3). The central role of Toll receptors as pattern-recognition receptors of the innate immune system has been established in the fly. They are essential for their reaction against fungal infection (31). *Drosophila* has a total of nine Toll receptors, but only one of them, Toll itself, has been shown to be relevant for immunity (6, 7). In the airway epithelium, *Toll*, *18-wheeler*, *Toll-7*, and *Toll-8* are expressed (13). Upon infection, the expression of Toll is increased in the airways (28), which is surprising because conventional Toll signaling is not functional in this tissue (13).

Deciphering the role of serine protease inhibitors (serpins) in asthma is another field where *Drosophila* may contribute to asthma research. One of these serpins, *SPINK5*, is an asthma-susceptibility gene. The initially reported association of genetic variants in *SPINK5* with atopic dermatitis comes from a population that was recruited through asthma cases. Our own investigations indicate that it is related to a special phenotype where asthma and atopic dermatitis are combined (32). A functional equivalent in *Drosophila* is the serpin *Spn77Ba*. This serpin is present in the fly's airway epithelium and it plays a central role as a gatekeeper of the epithelial innate immune system. *Spn77Ba* disruption leads to strong activation of the epithelial immune system but also to activation of systemic immunity, meaning that release of antimicrobial peptides from the fat body is triggered by activation of the Toll pathway (33). These results indicate that serpins might act as controllers of epithelial immunity, ensuring an immunological homeostasis. Deregulation of their activity may thus induce prolonged inflammation.

Another family of asthma-susceptibility genes is the signal transducer and activators of transcription (STATs). In the fly, JAK/STAT signaling is simpler than in vertebrates. In mammals, numerous ligands, receptors, JAK kinases and STATs are present (34), whereas in *Drosophila* only one receptor, one JAK kinase and only one STAT (STAT92) constitute this pathway (35). Insofar as *STAT6* is an asthma-susceptibility gene and *STAT3* is believed to play an important role in asthma pathogenesis, the presence of only one STAT in the fly (*STAT92*) provides a unique opportunity to study the physiological significance of this factor in the airways. All components of the pathway, including the ligand *unpaired* (*upd*), are expressed in airway epithelial cells. In addition, we have shown that STAT-dependent signaling is operational in defined parts of the airways, without pathogen contact (13). A more detailed analysis of JAK/STAT signaling in the airway epithelium of the fly has therefore great potential to provide new information about its role in health and disease of the airways.

As a further example, G-protein-coupled receptors implicated in asthma also have defined homologs in the fly. Most important may be the fly's homolog of the adrenergic receptor, called *oct2β*, which is expressed in the airway epithelium. It shares not only structural similarities but appears to mediate similar effects in the airways via similar second-messenger systems (36). Not only can the effects of different gene doses on the airways be examined, but the long-term use of adrenergics and their impact on the airway epithelia are also experimentally accessible, because these drugs can be delivered effectively via feeding.

Another group of asthma-susceptibility genes, the glutathione-S transferases (GST), presents completely different issues. GSTs are well characterized and they are believed to act as general antioxidants that detoxify xenobiotics and facilitate normal lung function when their expression is increased in response to different

types of stress (37). Nevertheless, we know little about their physiological role in the normal lung, and we also have limited experimental information about their significance in asthma development. However, all major GSTs known to be relevant for asthma have homologs in the fly, and they are expressed in the airways, allowing their functional analysis in this model.

In addition, the role of miRNAs for asthma is a currently emerging topic. Targets for miRNAs have been identified that show an association with the prevalence for the development of asthma (38). Furthermore, it has become apparent that miRNAs are of importance to the regulation of immune function, especially with respect to the lung (39). In *Drosophila*, this class of miRNAs is highly conserved and whole genome-covering studies have identified numerous miRNA target sites (40).

Hence, the *Drosophila* model is useful in the context of asthma candidate genes of known function as highlighted here, but its true potential lies in combining the *Drosophila* asthma model with the most powerful genetic tools of today: genome-wide association (GWA) studies and second-generation, whole-genome sequencing. With these new techniques, discoveries of novel disease-susceptibility loci, genes, and disease-associated gene variants will reach unprecedented momentum. Most of the genes located in susceptibility loci will be of unknown function and their involvement in asthma has not been documented. To make full use of the information buried in GWA studies, high throughput tools to study the basic role of genes in disease mechanisms are needed to channel further in-depth analysis of genes and genetic variants. Although genotyping and sequencing have made quantum leaps forward, functional genomics studies are still tedious, expensive, and time consuming. As such, there is the danger that we may overlook or misinterpret major parts of GWA data. Translation of these results into basic asthma research and clinical applicability is slowed down or hampered considerably at this stage.

Drosophila is a valuable tool to categorize putative candidate genes for further downstream functional analyses in vertebrates. It may be used to dissect the likelihood that individual genes in a gene cluster may contribute causally to an observed susceptibility signal, but moreover, it can be used to identify the relevance of a gene to a disease pathway, identify stimuli for gene regulation, tissue specificity of regulation, and gene-by-environment interactions. These potential applications are relevant for all complex diseases but especially so for lung diseases where the organ of interest is difficult to access. Using *Drosophila* models, preliminary experiments with other species (such as mice) may be reduced and experiments in higher organisms better focused. Using *Drosophila* in a systematic approach together with other models and tools seems most promising and may significantly reduce the turnaround time from genetic results into biological meaningful data.

If successful, using the *Drosophila* model to elucidate the role of genes involved in the GWA study of susceptibility signals from chromosome 17q21 (4) may develop into a textbook example demonstrating the power of the fly in asthma research. Currently, we have no information regarding the physiological significance of ORMDL-3, which is the best candidate within this cluster. In *Drosophila*, the ORMDL family is made of only a single member that is also expressed in the airways. Here, the entire arsenal of *Drosophila* genetics can be adopted to determine its role in cell biology and physiology, with a focus on airway physiology, including targeted knock-down or overexpression. Taken together, *Drosophila* may develop into a complementary model in asthma research. The inherent strengths of this system may open a novel field in asthma research that might enable us to understand, at least in part, the role of important asthma-susceptibility genes in detail.

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References

- Laitinen T, Rasanen M, Kaprio J, Koskenvuo M, Laitinen LA. Importance of genetic factors in adolescent asthma: a population-based twin-family study. *Am J Respir Crit Care Med* 1998;157:1073–1078.
- Kere J. Mapping and identifying genes for asthma and psoriasis. *Philos Trans R Soc Lond B Biol Sci* 2005;360:1551–1561.
- Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 2008;8:169–182.
- Moffatt MF, Kabisch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufer A, Rietschel E, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007;448:470–473.
- Finkelman FD, Wills-Karp M. Usefulness and optimization of mouse models of allergic airway disease. *J Allergy Clin Immunol* 2008;121:603–606.
- Lemaitre B, Hoffmann J. The host defense of *Drosophila melanogaster*. *Annu Rev Immunol* 2007;25:697–743.
- Govind S, Nehm RH. Innate immunity in fruit flies: a textbook example of genomic recycling. *PLoS Biol* 2004;2:E276.
- Broide DH, Lawrence T, Doherty T, Cho JY, McElwain K, McElwain S, Karin M. Allergen induced peribronchial fibrosis and mucus production mediated by I κ B kinase β -dependent genes in airway epithelium. *Proc Natl Acad Sci USA* 2005;102:17723–17728.
- Pantano C, Ather JL, Alcorn JF, Poynter ME, Brown AL, Guala AS, Beuschel SL, Allen GB, Whittaker LA, Bevelander M, et al. Nuclear factor-kappaB activation in airway epithelium induces inflammation and hyperresponsiveness. *Am J Respir Crit Care Med* 2008;177:959–969.
- Rühle H. Das larvale Tracheensystem von *Drosophila melanogaster* Meigen und seine Variabilität. *Zeit wissenschaftl Zool* 1932;141:159–245.
- Ghabrial A, Luschnig S, Metzstein MM, Krasnow MA. Branching morphogenesis of the *Drosophila* tracheal system. *Annu Rev Cell Dev Biol* 2003;19:623–647.
- Liu L, Johnson WA, Welsh MJ. *Drosophila* DEG/ENaC pickpocket genes are expressed in the tracheal system, where they may be involved in liquid clearance. *Proc Natl Acad Sci USA* 2003;100:2128–2133.
- Wagner C, Isermann K, Fehrenbach H, Roeder T. Molecular architecture of the fruit fly's airway epithelial immune system. *BMC Genomics* 2008;9:446.
- Ryder E, Ashburner M, Bautista-Llacer R, Drummond J, Webster J, Johnson G, Morley T, Chan YS, Blows F, Coulson D, et al. The DrosDel deletion collection: a *Drosophila* genome wide chromosomal deficiency resource. *Genetics* 2007;177:615–629.
- Bellen HJ, Levis RW, Liao G, He Y, Carlson JW, Tsang G, Evans-Holm M, Hiesinger PR, Schulze KL, Rubin GM, et al. The BDGP gene disruption project: single transposon insertions associated with 40% of *Drosophila* genes. *Genetics* 2004;167:761–781.
- Venken KJ, Bellen HJ. Transgenesis upgrades for *Drosophila melanogaster*. *Development* 2007;134:3571–3584.
- Venken KJ, He Y, Hoskins RA, Bellen HJ. P[acman]: a BAC transgenic platform for targeted insertion of large DNA fragments in *D. melanogaster*. *Science* 2006;314:1747–1751.
- Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 1993;118:401–415.
- Duffy JB. GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. *Genesis* 2002;34:1–15.
- Roman G, Endo K, Zong L, Davis RL. P[Switch], a system for spatial and temporal control of gene expression in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 2001;98:12602–12607.
- Kalidas S, Smith DP. Novel genomic cDNA hybrids produce effective RNA interference in adult *Drosophila*. *Neuron* 2002;33:177–184.
- McGuire SE, Le PT, Osborn AJ, Matsumoto K, Davis RL. Spatiotemporal rescue of memory dysfunction in *Drosophila*. *Science* 2003;302:1765–1768.
- Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblaue S, et al. A genomewide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* 2007;448:151–156.
- Feany MB, Bender WW. A *Drosophila* model of Parkinson's disease. *Nature* 2000;404:394–398.
- Matthews KA, Kaufman TC, Gelbart WM. Research resources for *Drosophila*: the expanding universe. *Nat Rev Genet* 2005;6:179–193.
- O'Keefe LV, Smibert P, Colella A, Chataway TK, Saint R, Richards RI. Know thy fly. *Trends Genet* 2007;23:238–242.
- Chintapalli VR, Wang J, Dow JA. Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. *Nat Genet* 2007;39:715–720.
- Wagner C, Isermann K, Roeder T. Infection induces a survival program and local remodelling in the airway epithelium of the fly. *FASEB J* 2009; (in press).
- Takehana A, Katsuyama T, Yano T, Oshima Y, Takada H, Aigaki T, Kurata S. Overexpression of a pattern-recognition receptor, peptidoglycan recognition protein-LE, activates *imd/relish*-mediated antibacterial defense and the prophenoloxidase cascade in *Drosophila* larvae. *Proc Natl Acad Sci USA* 2002;99:13705–13710.
- Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. *Nat Rev Mol Cell Biol* 2007;8:221–233.
- Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* 1996;86:973–983.
- Kabisch M, Carr D, Weiland SK, von Mutius E. Association between polymorphisms in serine protease inhibitor, *kazal* type 5 and asthma phenotypes in a large German population sample. *Clin Exp Allergy* 2004;34:340–345.
- Tang H, Kambris Z, Lemaitre B, Hashimoto C. A serpin that regulates immune melanization in the respiratory system of *Drosophila*. *Dev Cell* 2008;15:617–626.
- Shuai K, Liu B. Regulation of JAK-STAT signalling in the immune system. *Nat Rev Immunol* 2003;3:900–911.
- Arbouzova NI, Zeidler MP. JAK/STAT signalling in *Drosophila*: insights into conserved regulatory and cellular functions. *Development* 2006;133:2605–2611.
- Roeder T. Tyramine and octopamine: ruling behavior and metabolism. *Annu Rev Entomol* 2005;50:447–477.
- He JQ, Connett JE, Anthonisen NR, Paré PD, Sandford AJ. Glutathione S-transferase variants and their interaction with smoking on lung function. *Am J Respir Crit Care Med* 2004;170:388–394.
- Tan Z, Randall G, Fan J, Camoretti-Mercado B, Brockman-Schneider R, Pan L, Solway J, Gern JE, Lemanske RF, Nicolae D, et al. Allele-specific targeting of microRNAs to HLAG and risk of asthma. *Am J Hum Genet* 2007;81:829–834.
- Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, et al. Requirement of *bic/microRNA-155* for normal immune function. *Science* 2007;316:608–611.
- Stark A, Brennecke J, Russell RB, Cohen SM. Identification of *Drosophila* MicroRNA targets. *PLoS Biol* 2003;1:E60.

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