

Chapter 5

A *Drosophila* Asthma Model – What the Fly Tells Us About Inflammatory Diseases of the Lung

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Abstract Asthma and COPD are the most relevant inflammatory diseases of the airways. In western countries they show a steeply increasing prevalence, making them to a severe burden for health systems around the world. Although these diseases are typically complex ones, they have an important genetic component. Genome-wide association studies have provided us with a relatively small but comprehensive list of asthma susceptibility genes that will be extended and presumably completed in the near future. To identify the role of these genes in the physiology and pathophysiology of the lung, genetically tractable model organisms are indispensable and murine models were the only ones that have been extensively used. An urgent demand for complementary models is present that provide specific advantages lacking in murine models, especially regarding speed and flexibility. Among the model organisms available, only the fruit fly *Drosophila melanogaster* shares a comparable organ composition and at least a lung equivalent. It has to be acknowledged that the fruit fly *Drosophila* has almost completely been ignored as a model organism for lung diseases, simply because it is devoid of lungs. Nevertheless, its airway system shows striking similarities with the one of mammals regarding its physiology and reaction towards pathogens, which holds the potential to function as a versatile model in asthma-related diseases.

Asthma and Other Chronic Inflammatory Diseases of the Lung

Asthma is the most common chronic inflammatory disease of the lung. Hallmarks of the disease include airway obstruction, bronchospasm, wheezing, coughing and shortness of breath. Chronic asthma comprises complex remodelling processes of the airway contributing to the disease phenotype. Like most inflammatory diseases, asthma is a complex disease, meaning that different intrinsic and extrinsic factors contribute to pathogenesis. Intrinsic factors, meaning genetic susceptibility, are

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highly relevant for asthma development, because family and twin studies revealed that the risk of developing asthma is increased several fold in first-degree relatives of asthma patients (Holloway et al. 2008). A number of different asthma susceptibility genes have been identified by positional cloning as well as by candidate gene studies. The results of these studies have challenged the generally accepted view of asthma pathogenesis, leading to the suggestion that new and unexpected pathways are involved in asthma development, which would substantially increase the number of potential targets for pharmacological intervention (Holgate and Polosa 2008). The large number of genome-wide association studies performed with large and well-defined cohorts of patients that are currently being carried out will provide us with an almost complete set of asthma susceptibility genes. The usefulness of this rich source of information is currently limited by our inability to understand the connection between genes and disease development, at least for the majority of them. In asthma, the situation is obviously similar to all other complex diseases: the identification of susceptibility genes is the beginning and not the end of the story. To profit from these rich sources, model organisms are of paramount importance to elucidate the relevance of these genes and the genetic variations identified. Transgenic mice, including “humanized” mice, are currently the sole genetically tractable models used successfully in asthma research (Finkelman and Wills-Karp 2008). However, some drawbacks of these models hinder progress in our understanding of the disease. Although they are very useful, transgenic and mutant mice have drawbacks that obscure a direct analysis of the expected phenotypes. Genetic redundancy and developmental plasticity are especially important issues that often impair the direct identification of gene function. In addition, it is a complex task to produce mice tailored to the needs of lung physiologists, because the lung is a very complex organ with numerous resident as well as motile cell types. To supplement this technical resource, other animal models, especially those, which have a far lesser complexity, would greatly facilitate research in this area.

***Drosophila* as a Model in Biomedical Research**

Developments in recent years revealed that biomedical research critically depends on suitable animal models to understand the molecular basis underlying the pathogenesis of human diseases and to provide systems for developing and testing new therapies. Despite the supremacy of murine models, other model organisms are able to provide new and relevant information. Model organisms have to fulfil some criteria including sequenced genomes, short life cycles, similarities with human genes/proteins, and the ease of genetic manipulation, which is of prime importance. Among the limited number of well-established and generally accepted model organisms (yeast, *C. elegans*, *Drosophila*, zebra fish, and mice), the fruit fly *Drosophila* is the *primus inter pares*. It is the oldest model organism and was introduced almost a century ago by Thomas Hunt Morgan. Sequencing and analysis of its genome revealed a completely unforeseen degree of similarity with our own genome. More than 60% of all human disease genes have homologous counterparts in the fly (Fortini et al. 2000), which led to the development of a special database listing all these candidate genes (Chien et al. 2002). Since then, *Drosophila* models for a large number of different human diseases have been developed (Bier 2005). Among the first ones were those focusing on neurodegenerative diseases (Feany and Bender 2000). In addition to this Parkinson model, very informative models of Huntington’s and Alzheimer’s disease have been established (Chan and Bonini 2000), which triggered a great number of follow-up studies, finally leading to new therapeutic ideas. The plethora of models based on the fruit fly comprises those for the analysis of cardiac diseases (Wolf et al. 2006) or diabetes (Baker and Thummel 2007) to mention only two out of a huge number. For the unprejudiced reader it may be hard to understand why *Drosophila* should be that well suited for the study of human disease as

the anatomical organization of the fruit fly is much simpler than that of mice. The major question is what makes *Drosophila* so special? *Drosophila* is simple enough to be a highly facile model but at the same time, major organs, physiological processes, and behaviours are similar to those found in higher mammals. This makes comparison between humans and flies much more relevant than comparisons between humans and worms or between humans and yeast. Together with the vast amount of knowledge that has been accumulated during the last century and the availability of countless technical opportunities to manipulate the fly, *Drosophila* is a model organism beyond comparison.

In the last couple of years, an incredibly rich source of tools has been developed allowing the study of the functional significance of genes of interest. Numerous deletions and far more than 12,000 transposable element insertions are readily available for creating genomic mutations. Transgenesis is very easy and there are a number of corresponding vectors available and some of them have been developed that capitalize on *P* element, ϕ C31 and recombineering technologies (Bellen et al. 2004; Venken and Bellen 2007; Venken et al. 2009). The most widely used and presumably the most versatile tool is the binary GAL4/UAS-system invented more than 15 years ago (Brand and Perrimon 1993). It relies on the yeast transcription factor GAL4 and the corresponding DNA binding region UAS that constitutes an extremely useful construction kit. Gal4/UAS systems conveniently allow overexpression or silencing of genes of interest wherever the researcher requires it. Both components (Gal4 and UAS) are segregated into different fly lines, called driver and effector lines, respectively. Effector lines (UAS-lines) determine what is expressed and driver lines (Gal4-lines) determine the stage of development and location of expression. Countless different driver (Gal4) lines are available, enabling the researcher to address almost every fly tissue separately (Fig. 5.1). Similarly, a great number of effector lines are available or can be generated easily by producing corresponding flies carrying a construct in which, for example, overexpression of a certain gene can be achieved because it is under transcriptional control of particular UAS elements. Crossing these two types of lines activates this system in the entire F1 generation (Fig. 5.1). This simple construction kit allows design of numerous different experiments with a small number of fly stocks, simply by means of combination. As the parental strains are usually not impaired, researchers can induce silencing of various genes of interest in one organ or overexpression of another gene in a different set of organs simply by crossing the corresponding lines. To complement this armamentarium, Barry Dickson's group completed the great endeavor of producing RNAi lines for almost all annotated *Drosophila* genes (Dietzl et al. 2007), and made them accessible to the *Drosophila* community. 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 that is utilized. This unique resource enables even large-scale studies at reasonable prices and in a reasonable period of time.

A recent very useful refinement of this method allows additional temporal control of expression. The so-called TARGET system (McGuire et al. 2003) includes a temperature-sensitive repressor of GAL4, referred to as GAL80ts. It represses expression under restrictive conditions (19°C). A shift 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. Usually Gal80ts is expressed under control of the ubiquitously active tubulin promoter, thus enabling effective inhibition in all organs at almost all time points.

More complex experiments became possible by the addition of alternative binary expression systems, namely the *lexA*-based binary expression system and the so-called Q-system (Potter et al. 2010). Many other powerful techniques exist that maximize the abilities of what can be studied in the fly. It is impossible to summarize all of them, but the reader is referred to additional reviews for more detailed discussion of recent developments in the field of *Drosophila* genetics (Bellen et al. 2004; Venken and Bellen 2007; Venken et al. 2009).

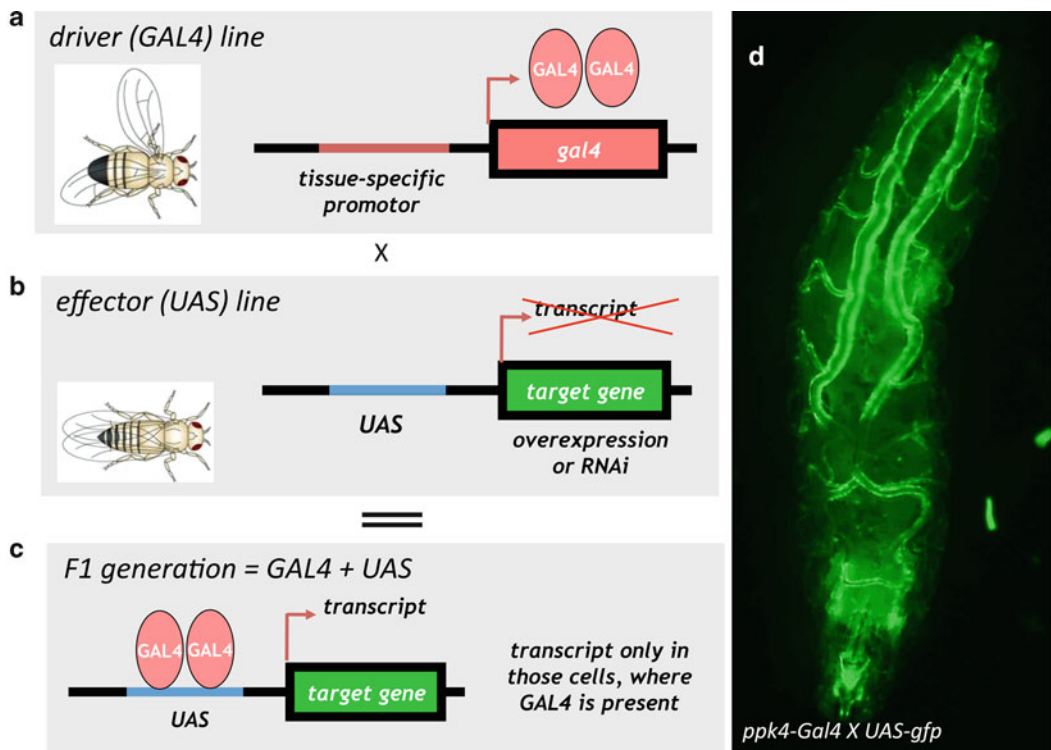


Fig. 5.1 Binary expression control systems allow targeted manipulation in the fly. A number of different binary expression systems are available for the fly. Most important is the Gal4/UAS system derived from yeast. Here, a so-called driver or Gal4 line determines the temporal and spatial expression pattern via expression of the yeast transcription factor Gal4 (a). A second type of line, the so-called effector or UAS-line has the target gene under transcriptional control of the UAS enhancer, which is also borrowed from yeast. This one is activated by Gal4 only (b). In the F1 generation, both factors (Gal4) and the UAS enhancer come together to direct expression of the target gene in a manner controlled by the expression pattern of the Gal4 line (c). Using an airway epithelium specific driver line (ppk4-Gal4) and gfp as the effector, we could label the airways epithelium specifically (d)

The Airway System of the Fly

Regarding asthma, the airway epithelium is obviously the most important target. Flies do not have lungs, but a tracheal system that supplies the entire organism with oxygen. Although lungs and tracheal systems are not homologous, a huge number of similarities can be observed at the level of the most important cell type comprising the corresponding organs, namely the airway epithelial cell. In *Drosophila*, the airway epithelium is a single cell layer surrounding the central airway. The airway system, or the tracheal system is made of interconnected tubes starting from major branches to secondary ones and ending in terminal branches supplying almost every cell of the fly (Fig. 5.2). Contact to the outside world is made through spiracles, which ensure an optimal oxygen flow (Affolter et al. 2003; Ghabrial et al. 2003; Ruehle 1932; Whitten 1957).

Important to note is that the airway system of the fly is made of epithelial cells only, meaning that no other cell types confound signals from these cells during experimental analysis.

The airway system is able to react towards an infection with an epithelial immune response, carried out by the immune competent epithelial cells. All airway epithelial cells have this capability, which is mainly characterized by the expression of antimicrobial peptide genes (Wagner et al. 2008).

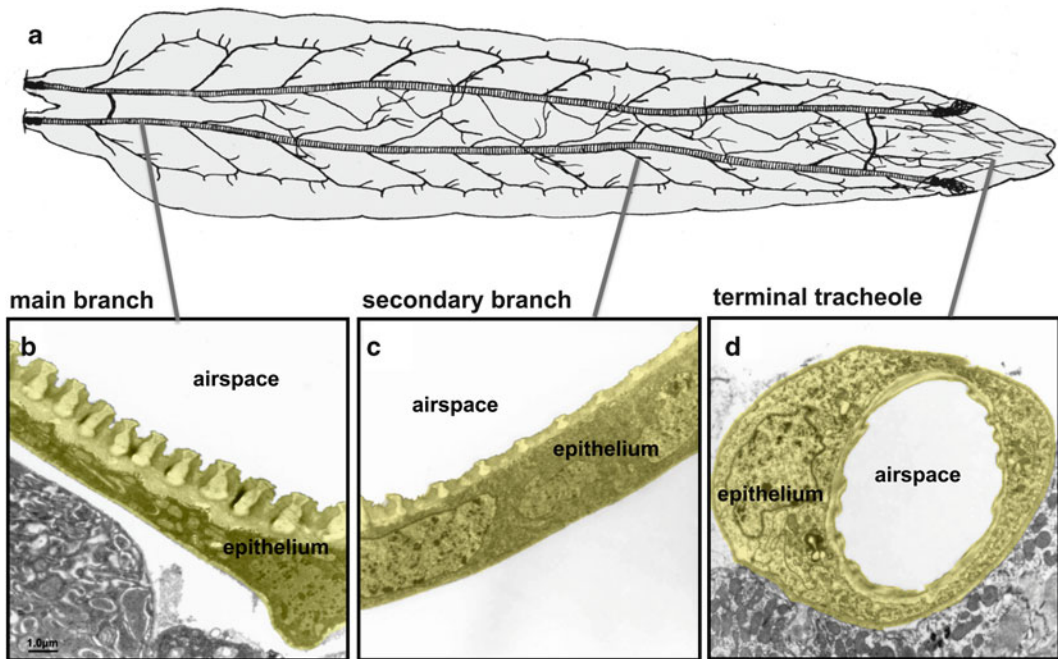


Fig. 5.2 The airway system of the fly has a very simple organization. It is composed (here a schematic drawing of a larva; **a**) of interconnected tubes that are organized in a hierarchical order. These tubes contain a central air filled lumen that is enclosed by a single layered epithelium. This organization is seen throughout the entire system ranging from the primary branches (**b**), including the secondary ones (**c**) and ending in the terminal tracheoles that supply all cells of the body with oxygen (**d**)

A detailed analysis of the signalling pathways present in the fly's airway epithelium and the pattern recognition molecules revealed that only one pathway converging on NF- κ B activation, the IMD pathway, is operational in these tissues. In addition, the majority of pattern recognition molecules that the fly has to its disposal are present and functional there. It should be mentioned that other signalling pathways with relevance for immune reactions, including the JAK/STAT- and JNK-pathway are also functional in these cells (Wagner et al. 2008). All aspects of adaptive immunity are absent in the fly, making an unveiled view to the innate immune system possible, that shares most structural and architectural features with our own. Other physiological similarities include the way liquid clearance is achieved, just to mention one among a large number of examples.

Manipulation of the Airway Epithelium of the Fly

The airway system of the fly has been one of the prime models to study basic mechanisms underlying angiogenesis and comparable developmental traits. A huge number of studies have been performed with the goal to elucidate the mechanisms underlying tissue formation and development of complex structures. As these studies have almost completely been restricted to embryogenesis, the airway system of larvae and adults is much less well understood. Nevertheless, these studies have yielded a great number of highly relevant tools with which the airway system can be manipulated. Most important is obviously the Gal4/UAS system that allows all kinds of manipulation. To gain access to this enormously rich armament of possibilities, Gal4 lines that exclusively address the

airway epithelium are mandatory. In larvae, we have some of these lines to our hand, namely *btl-Gal4* and *ppk4-Gal4*. Both are exclusively expressed in the airway epithelium, without any confounding signal in any other organ. Unfortunately, this very promising situation is currently restricted to work with larvae, as the correspondingly specific drivers for the adult airway system are not available yet.

Thus, most of this work that depends on the advantages of the *Drosophila* system is currently performed with larvae, which on the other hand has the great advantage that the airway system is easily accessible to all kinds of microscopic analyses even in the living animal.

***Drosophila* in Asthma Research**

The fruit fly has gained acceptance as a model organism to study aspects of human diseases, but asthma is surely one disease that doesn't initially spring to mind. Flies lack most characteristics that are thought to be highly relevant for asthma, such as adaptive immunity including IgEs and T-cells. In addition, outcomes of the disease such as wheezing and airway hyper-responsiveness have not been observed in flies. However, insights gained in the last few years have dramatically changed the way asthma is viewed – from a disease that is strictly dependent on the adaptive immune system only, to one where the innate immune system and the epithelial cells are of prime importance (Hammad et al. 2009; Holgate 2007). Apparently, epithelial cells orchestrate most adverse effects associated with this disease in a yet not well-understood way. Another central aspect relevant for asthma development is the innate immune system and especially the innate immune system within the airway epithelial cells. It became clear that those innate immune signaling pathways that converge on activation of NF- κ B factors are of central importance for various aspects of disease development and progression (Broide et al. 2005; Pantano et al. 2008). Both of these newly identified hallmarks of asthma pathogenesis, the involvement of the innate immune system and NF- κ B, brought the fly back into focus as a relevant model, simply because their contribution to disease development can be studied easily in the fly (Lemaitre and Hoffmann 2007; Roeder et al. 2009). Parallel to these developments, another set of asthma-related research also significantly changed the way the disease is viewed. Genetic research has revealed a number of asthma susceptibility genes (Vercelli 2008). In addition, with the development of genome-wide association studies, the roles of many more candidate genes in asthma will be uncovered. Surprisingly, most of these asthma susceptibility genes have nothing to do with adaptive immunity. Instead they are associated with innate immunity and with ways of ensuring epithelial integrity. Susceptibility genes that can be classified into either of these categories (innate immunity and epithelial integrity) comprise approximately 60% of the total. Except for those associated with adaptive immunity, we and others found unequivocal orthologues for all the rest (Roeder et al. 2009). Most asthma-related genes are expressed in the airway epithelium of the fly. Even more interestingly, some of them 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. Indeed, what was previously thought to be a major disadvantage of flies for asthma research, the absence of adaptive immunity, may actually be a significant advantage for the researcher, because it allows an exclusive view on the role of innate immunity under different physiological conditions. In mice, it is a very complex task to distinguish between responses of the adaptive and the innate immune system, because they are highly interconnected.

Two different complementary experimental approaches are used in asthma-related *Drosophila* research. The first one is based on the observation that manipulation of innate immune systems within the epithelial cell is sufficient to induce structural changes very similar to those observable in chronic asthma patients or in murine models of the disease. The other approach depends on use

of the *Drosophila* model for elucidation of the physiological as well as the pathophysiological significance of asthma susceptibility genes.

The first route of studies was initiated earlier in our lab and is based on the observation that the epithelial immune system of the fly's airway epithelium responds to an infection with bacteria or fungi not only with the expression of anti-microbial peptide genes, but additionally shows other reaction phenotypes that are relevant for asthma development. These phenotypical changes occur after prolonged and very strong activation of the epithelial immune response. Those parts of the tracheal system that react most strongly to an airway infection show significant airway remodelling. The remodelling of airways is one hallmark of chronic asthma. In the fly, the most conspicuous sign of remodeling is a several fold increase in epithelial thickness. This is accompanied by the production of new airway epithelial cells, something that usually does not occur during normal larval development in the fly. Taken together, these cells become meta- and hyperplastic, thus showing hallmarks of airway remodeling upon a prolonged strong activation of this (very special) type of immune system (Wagner et al. 2009). One major reason for this unprecedented type of reaction may be that these cells forcefully try to obviate apoptosis. Following infection highly potent apoptosis-inducing genes are activated, including the autophagy-specific kinase 1 gene (Atg1), whose overexpression itself is sufficient to induce apoptosis. Activation and overexpression of dFoxo appears to be a way to enable survival under these hostile conditions, which in turn, may be the major reason for the observed phenotypic variations (Wagner et al. 2009).

The second experimental route is a straightforward one, taking advantage of the entire toolkit available in *Drosophila* genetics. As already mentioned, more asthma susceptibility genes are involved in innate immune responses or in maintaining epithelial cell integrity than in processes associated with adaptive immunity. Innate immune signalling within these epithelial cells is necessary and sufficient for experimental disease induction, either in mice or flies. The central position of epithelial cells for orchestration of for example airway remodeling has been emphasized in recent physiological studies.

To show that *Drosophila*, with all its advantages, can be used as a model for asthma research, we analyzed the canonical set of asthma susceptibility genes and tried to find homologues in the fly (Roeder et al. 2009). Some of these genes, associated with adaptive immunity, have no counterparts in the fly. These genes include: IL-4, IL-10, IL-13, IL-18, IL-4R, CC16, CTLA4, HLA-DBP1, HLA-DQB1, HLA-DRB1, LTA, LTC, and FCERIB. For all other candidates, we found orthologues in the fly genome. This is summarized in Table 5.1. Most of them are indeed expressed in the fly's airway system and some of them (the majority) are regulated upon infection or ectopic manipulation of the IMD-signalling pathway. For most of these *Drosophila* counterparts, genetic tools are 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 5.1). Obviously, the methods to manipulate the airway system of the fly or orthologues of asthma susceptibility genes within this tissue are numerous, but what about simple read-outs? 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, which are often caused by liquid-filled parts of the tracheal lumen, are easily identified. A physiological test of the operational capability of the tracheal system takes advantage of the normal behaviour of the larvae. Healthy animals are usually buried into their food, but upon functional oxygen deprivation, they move to the surface. This is also observed in larvae that show impairments of the tracheal system. This behaviour can be quantified (time to leave food or percentage of larvae showing this behaviour). The reason for choosing a seemingly simple model organism is that one hopes to be confronted with a significantly reduced complexity of the genomic situation, meaning much lesser redundancy (number of genes of a certain gene family) and much lesser plasticity (meaning that genes can take the role of a deleted one). Both aspects are obviously on the side of the fly. Usually, gene families that

Table 5.1 Asthma susceptibility genes and their homologues in *Drosophila*

Asthma gene	Drosophila homologue	Expressed in airways	Regulated or relevant in airways	Genomic resources
ACE	ANCE-family	+	ance-2	RNAi
ADAM33	Neu3(CG7649), mmd(CG42252)	?,?		all:KO, RNAi
ADRB2	Oct2B-like	+	Oct2B-like	KO, RNAi
CCL11,CCL5	upd1-3	+,+,?	upd-2	KO, RNAi
CD14	CG5195	?		RNAi
CMA1	trypsin29F,snk	?,+		RNAi;KO, RNAi
DPP10	ome,CG17684	?,+		both:KO, RNAi
FLG	CG1433 Atu	?		KO, RNAi
GPRA	CcapR,AICR2, TakR86C	?,+,+	AICR2	all:KO, RNAi
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
MMP9	DmMMP1	+	DmMMP1	KO, RNAi,OE
NAT2	aaNAT-1	?		KO, RNAi
NOS	dNOS	+		KO, RNAi,OE
ORMDL3	dORMDL	+		RNAi
STAT6	STAT92	+	STAT92	KO, RNAi,OE
SPINK5	Serpins	+	spn77Ba	KO, RNAi
TBXA2R	CG7497,OAMB	?,+		KO, RNAi; KO, RNAi,OE
TGFb1	activin, maverick, gbp	?,?,?		RNAi, -; RNAi
TLR2, 4, 6, 10	Toll receptors	+	Toll, 18-w, Toll-7, -8	all: KO, RNAi
TNF	Eiger	?		RNAi

Listed are the orthologues of canonical asthma susceptibility genes as well as the presence and regulation in the airway epithelium. In addition, the available genomic resources have been extracted from the VDRC (Dietzl et al. 2007) or flybase (www.flybase.org).

have numerous members in mammals are comprised 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 in *Drosophila* only two MMPs are present and only one of them is expressed in the airway epithelium (Page-McCaw et al. 2003).

Potential Roles of Asthma Susceptibility Genes in *Drosophila*

To show the potential of the *Drosophila* system, we would like to discuss our current state of knowledge regarding some of the homologues of asthma susceptibility genes. Among the matrix metalloproteinases (MMP), at least two, namely MMP9 and MMP7, are considered as highly relevant for asthma (Table 5.1). Using the sole airway epithelium relevant homologue found in *Drosophila* (DmMMP1), their role could be studied in order to tease apart its function independent of the other 21 MMPs (Goswami et al. 2009). Nevertheless, we have almost no experimentally based information regarding its direct role in asthma development and progression. In *Drosophila*, DmMMP1 has been shown to be important for tracheal development (Page-McCaw et al. 2003).

DmMMP1 and MMP9 are both secreted and they accept collagen as a substrate, pointing to similar functions. This suggests that a closer look at the physiological role of DmMMP1 in airway biology using the methodological toolkit available in *Drosophila* will allow us a better understanding of MMP9's and MMP7's contribution to asthma pathology.

Another important group of asthma susceptibility genes are the signal transducer and activators of transcription (STATs). In the fly, the complexity of JAK/STAT signalling is reduced to its minimum. In mammals, various families of ligands, receptors, JAK kinases and STATs are present, whereas in *Drosophila* only one receptor, one JAK kinase, and one STAT constitute this pathway (Arbouzova and Zeidler 2006; Shuai and Liu 2003). Since both, *STAT6* and *STAT3* are believed to play important roles in asthma pathogenesis, the presence of only one STAT homologue in the fly 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 signalling is operational in defined parts of the airways, even without pathogen contact (Wagner et al. 2008). A more detailed analysis of JAK/STAT signalling 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.

Another class of genes that appears to be relevant for asthma pathogenesis also has some members in the fly. These are the serpins (serine protease inhibitors) and one member, namely *SPINK5*, is a well-established asthma susceptibility gene. The copious number of genes with seemingly very similar functional roles complicates understanding the physiological and pathophysiological role of serpins. A functional equivalent in *Drosophila* is the serpin *Spn77Ba*. This serpin is present in the fly's airway epithelium, where it plays an important role as a gatekeeper of the epithelial innate immune system. Its disruption induces strong activation of the epithelial but also of the systemic immune system. The latter response appears to be mediated by an unknown pathway, finally leading to release of anti-microbial peptides from the fat body, which is triggered by activation of the Toll pathway (Tang et al. 2008). This implies that serpins act as controllers of epithelial immunity, ensuring that immune-relevant proteases are inhibited in times of normal activities. Their deregulation may induce prolonged inflammation and thereby contribute to asthma development and chronification.

Completely different types of molecules that are highly relevant in asthma are G-protein coupled receptors as major transducers of information. Adrenergic receptors are of special interest, because they are targeted by a huge number of asthma-related pharmaceuticals. With respect to this, the most important may be the fly's orthologue of the adrenergic receptor, called *oct2 β* , 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. The methods available to directly manipulate second-messenger systems in the airway epithelia can be used to study the effects of long-term medication with adrenergics, an aspect that is not understood at all.

The last group of genes that should be discussed are the glutathione-S transferases (GST), which is a very copious gene family in our genome. Some of them are asthma susceptibility genes. They are believed to function as general enzymatic antioxidants that detoxify xenobiotics and facilitate normal lung function when their expression is increased in response to different types of stress. Surprisingly, we know almost nothing 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 pathogenesis have orthologues in the fly. All these orthologues are expressed in the airways, allowing their functional analysis in *Drosophila*. The full usefulness of the *Drosophila* model may emerge in combination with the most powerful genetic tools of today: genome-wide association studies (GWAS) and second generation, whole genome deep sequencing. These new techniques promise that discoveries of novel disease susceptibility loci, genes and disease-associated gene variants will reach unprecedented speed. As most of the genes

located in susceptibility loci will presumably be of unknown function and their involvement in asthma will not have been documented, a quick but comprehensive test regarding their potential role in airway physiology and pathophysiology is mandatory. While 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 major parts of genome-wide association studies. Thus, translation of these results into basic asthma research and clinical applicability may be slowed down or hampered considerably at this stage.

Drosophila is thus a valuable tool that might enable us to categorize putative candidate genes for further downstream analyses in vertebrate models, including murine models. *Drosophila* may be used to dissect the fraction by which individual genes of a gene cluster contribute causally to an observed susceptibility signal. Even more importantly, it can be used to identify the relevance of a gene in a disease pathway or identify stimuli relevant for gene regulation. These potential applications are relevant for all complex diseases but especially for lung diseases such as asthma, because the mammalian organ of interest is difficult to access and resident as well as motile elements contribute to disease development and progression. Using *Drosophila* models, preliminary experiments with other species (especially with mice) may be reduced significantly, allowing a better focus for these time-consuming studies. Utilizing *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 biologically meaningful data.

Taken together, *Drosophila* has the potential to develop into a complementary model in asthma research. The inherent strengths of this system can 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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