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AT A GLANCE

SUBJECT COLLECTION: TRANSLATIONAL IMPACT OF *DROSOPHILA*

Drosophila tools and assays for the study of human diseases

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ABSTRACT

Many of the internal organ systems of *Drosophila melanogaster* are functionally analogous to those in vertebrates, including humans. Although humans and flies differ greatly in terms of their gross morphological and cellular features, many of the molecular mechanisms that govern development and drive cellular and physiological processes are conserved between both organisms. The morphological differences are deceiving and have led researchers to undervalue the study of invertebrate organs in unraveling pathogenic mechanisms of diseases. In this review and accompanying poster, we highlight the physiological and molecular parallels between fly and human organs that validate the use of *Drosophila* to study the molecular pathogenesis underlying human diseases. We discuss assays that have been developed in flies to study the function of specific genes in the central nervous system, heart, liver and kidney, and provide examples of the use of these assays to address questions related to human diseases. These assays provide us with simple yet powerful tools to study the pathogenic mechanisms associated with human disease-causing genes.

KEY WORDS: *Drosophila*, Human disease models, Nervous system, Neurodegeneration, Regeneration, Heart, Liver, Oenocyte, Fat body, Kidney, Nephrocytes, Malpighian tubules

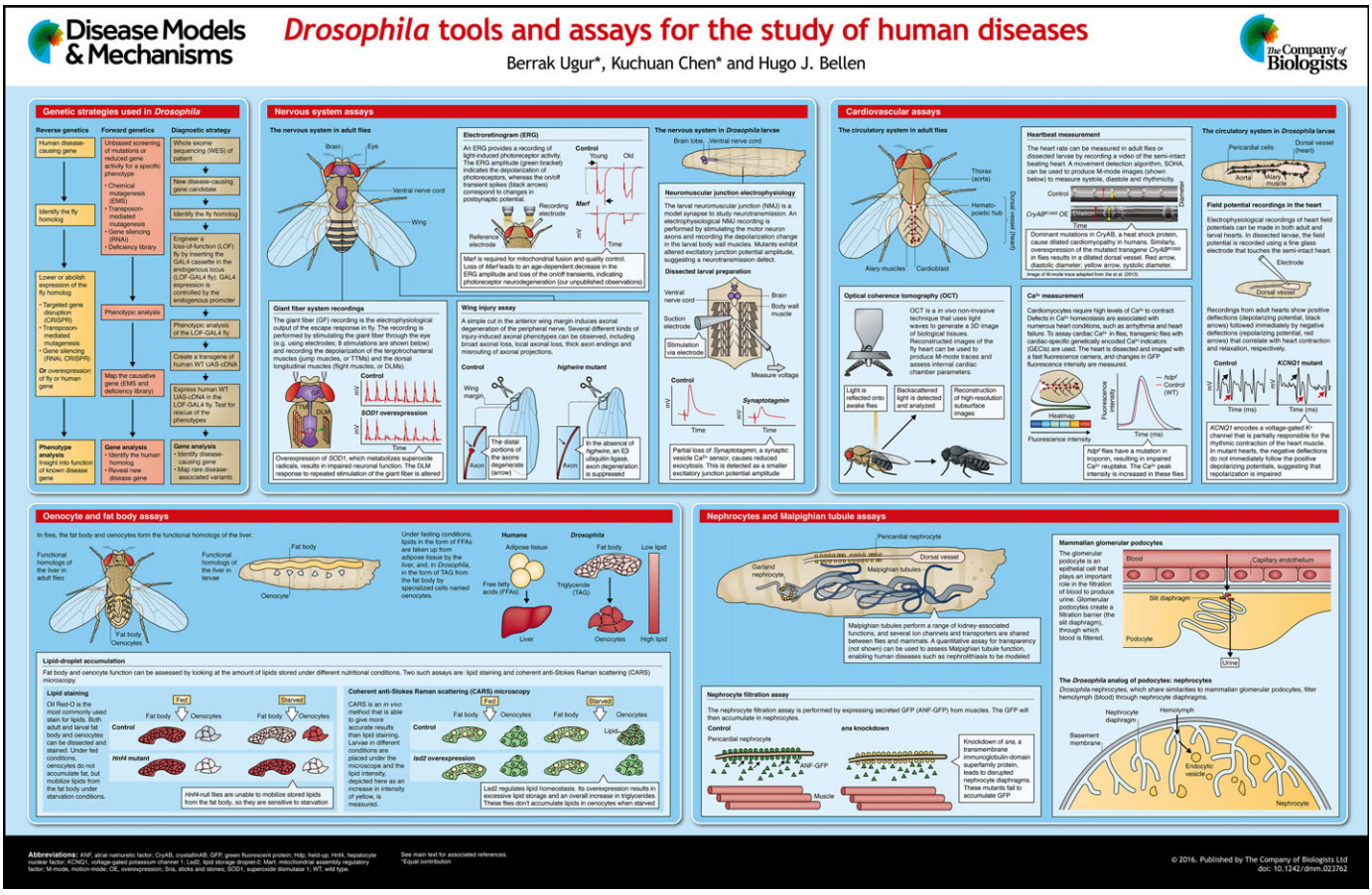
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Introduction

The fruit fly has come a long way since Charles W. Woodworth, an American entomologist, first proposed to use *Drosophila melanogaster* as a genetic model organism in 1900 (Sturtevant, 1959). In the past 100 years, fly research has been particularly



Disease Models & Mechanisms

Disease Models & Mechanisms (2016) 9, 235-244 doi:10.1242/dmm.023762

valuable for the analysis of molecular mechanisms underlying genetic phenomena, behavior and development. Approximately 65% of human disease-causing genes are believed to have a functional homolog in flies (Chien et al., 2002; Yamamoto et al., 2014) and a significant fraction of these homologs are expressed in *Drosophila* tissues that perform the function of the equivalent human tissue (Chintapalli et al., 2007). In our opinion, the evolutionary conservation of genes and their associated functions has not yet been exploited to its full potential, particularly in translational biology. Given the genetic tractability of flies and the many tools available for their genetic manipulation, numerous studies can now be performed in flies to more rapidly discover the molecular mechanisms by which human mutations cause disease phenotypes.

Broadly speaking, three main strategies to study human diseases using fly models have been developed: (1) reverse genetics, (2) forward genetics and (3) a recently established strategy to aid in the discovery of human disease-causing genes, which we name 'diagnostic strategy' (see the first panel in the poster). In the reverse genetics approach, mutations are created in fly homologs of human genes to study their phenotypes *in vivo*. There are mainly three ways to lower or abolish expression of a gene in flies; targeted gene disruption [e.g. using clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9)] (Beumer and Carroll, 2014), transposon-mediated mutagenesis and excision of existing transposable elements (TE), and gene silencing [via RNA interference (RNAi), CRISPR] (Mohr, 2014). In addition to loss-of-function studies, a wild-type or mutant version of a human disease-causing gene (transgene) can be overexpressed in flies to assess the effects in specific tissues (Feany and Bender, 2000).

In forward genetics, mutations are induced at random and the animals are screened for a particular phenotype. Mutations can be generated chemically [e.g. using ethyl methanesulfonate (EMS)] or by using transposons (Venken and Bellen, 2014), or mutants can be isolated by screening an RNAi library or a collection of existing deficiencies (Cook et al., 2012). This is an unbiased strategy that can help identify uncharacterized mutations in known disease genes (phenotypic expansion) as well as genes that have not been previously linked to disease. Forward genetics can thus be a powerful driving force for identifying previously unknown genes and unraveling biological phenomena.

Lastly, through the diagnostic strategy, *Drosophila* can be used to assess the pathogenic properties of rare variants that have been linked to human diseases. There are many United States (USA)-based initiatives designed to identify human disease-causing genes by sequencing the whole exome or genome of patients and their siblings or parents, coordinated by e.g. Centers for Mendelian Genomics (http://www.mendelian.org/) and the Undiagnosed Diseases Network (UDN; http://undiagnosed.hms.harvard.edu/). Similar strategies have been adopted in other countries, including the UK (http://www.uk10k.org/), China (Guangzhou *Drosophila* Resource Center and the Center for Genomic Sciences in the University of Hong Kong), and many more. This strategy is often inadequate to determine the causative gene variant when three or fewer individuals are assessed. We developed a pipeline that overcomes this challenge and enables the causative mutation to be pinpointed. First, the fly homolog or ortholog is knocked out by integrating a *GAL4* gene under the control of the endogenous regulatory elements, and the phenotype is assessed. If this phenotype can be rescued by expression of the wild-type *UAS*–human-cDNA but not by the human variant, causality is determined (Bellen and Yamamoto, 2015; Wangler et al., 2015).

In the following sections we will describe assays designed for key *Drosophila* organ systems that permit the study of homologs of human genes that cause disorders affecting the nervous system, heart, liver and kidney. We refer readers to other reviews focused on the fruit fly tracheal system (Wagner et al., 2008), peripheral nervous system (Charng et al., 2014) and gut (Apidianakis and Rahme, 2011), because these organ systems are not discussed here.

Nervous system assays

The nervous system in *Drosophila* is required for sensing and processing information related to vision, hearing, olfaction, proprioception and taste. Just like in humans, this information is conveyed to the CNS and processed to provide a motor output. Although the gross anatomy of *Drosophila* and human brain is very different, they share numerous conserved genetic, cellular, electrophysiological and chemical properties. As in vertebrates, many different types of neurons are required to process information in fruit flies. For example, in the visual system, ∼115 different types of neurons have been identified in *Drosophila*, which is a very similar number to what has been estimated in vertebrates (Venken et al., 2011). However, there are probably a million-fold fewer neurons overall in flies than in vertebrates. The reduced complexity and ease to study the *Drosophila* nervous system allows an in-depth assessment of the function of genes and neuronal networks. Many different assays have been developed to assess neuronal function. These include hearing, flight, learning and memory, and diurnal rhythmicity assays, as well as numerous highly specific behavioral assays (Branson et al., 2009; Inagaki et al., 2010; McGuire et al., 2005; Simon and Dickinson, 2010). In the last two decades, *Drosophila* has been increasingly used to model neurological dysfunction, including neurodegeneration, epilepsy, dementias, stroke, traumatic brain injury and brain tumors. In this section, we provide a snapshot of the different assays that can be used to study neurological disorders and describe the specific contexts in which they have been most useful so far. These tools allow researchers to gain novel insights into pathogenic mechanisms and might help to provide new therapeutic strategies for different neurological diseases.

Electroretinogram

Unlike vertebrate photoreceptors (PRs), which are light-sensory cells in the retina that connect to a neuron, fly PRs themselves are light-sensory neurons that project axons into the deep lamina and medulla layers of the adult brain. The ease of accessibility of fly PRs and their regular arrangement have facilitated the development of several assays to study retinal disorders and other more general neurological phenotypes. The electroretinogram (ERG) records the change in electrical activity of the PRs in response to a flash of light. The ERG depolarization amplitude provides a read-out of the phototransduction process, whereas the on/off transient spikes at the onset and offset of a light flash correspond to postsynaptic potential changes (see poster) (Hardie and Raghu, 2001; Stark and Wasserman, 1972).

ERG recordings were adapted to assess mutant phenotypes for *Drosophila* in the late 1960s (Hotta and Benzer, 1969; Pak et al., 1969). Given the ease of the assay, forward genetic screens based on ERGs in homozygous viable mutants permitted the identification of numerous genes that control the phototransduction pathway (Wang and Montell, 2007). Impaired phototransduction results in aberrant ERG recordings; for example, reduced depolarization amplitude and/or loss of the on and off transients (see example in the poster). Most of the mutations that affect the phototransduction pathway do not affect viability because the eye is not an essential organ in the

Disease Models & Mechanisms (2016) 9, 235-244 doi:10.1242/dmm.023762

fly. To circumvent the issue of lethality and study the role of essential genes in the eye, a technique was developed to generate eye-specific mosaic clones (Newsome et al., 2000; Stowers and Schwarz, 1999). This allowed the identification of genes that are more broadly implicated in neurodegeneration by measuring ERGs in young and old animals to document the time-dependent change in neuronal function (Haelterman et al., 2014; Yamamoto et al., 2014). Using this approach, the Bellen lab identified several fly mutants in which the human homologs have been implicated by other studies to play a role in various neurodegenerative diseases, including Leigh syndrome (*C8orf38/sicily*) (Zhang et al., 2013b), Charcot-Marie-Tooth disease type 2A (*MFN2/Marf*) (Sandoval et al., 2014) and autosomal recessive spastic ataxia with leukoencephalopathy (*MARS2*/*Aats-met*) (Bayat et al., 2012). In addition, we recently determined that reactive oxygen species (ROS) accumulate in the PRs of these mutants and, in turn, this triggers lipid-droplet formation that eventually contributes to PR neurodegeneration (Liu et al., 2015). These examples illustrate how ERGs can be effective tools to identify genes and elucidate molecular mechanisms underlying neurodegeneration.

Neuromuscular junction electrophysiology

The neuromuscular junction (NMJ) is the connection (synapse) between the motor neuron and the muscle. Disorders of the NMJ span a variety of phenotypes and can be due to genetic or acquired causes (Rodríguez Cruz et al., 2014). The *Drosophila* NMJ provides a powerful platform to study neuromuscular diseases because it allows detailed analyses of structural connections between the neuron and the muscle as well as their electrophysiological properties. The fly larval NMJ consists of arrays of overlapping striated muscle fibers that are innervated by motor neurons that form synaptic boutons (Prokop and Meinertzhagen, 2006). The fly NMJ is a large glutamatergic synapse that is easy to access, thereby permitting a detailed characterization of the properties of synaptic transmission, including assessment of excitatory junctional potentials (EJPs), spontaneous miniature EJPs, synaptic plasticity, transmission electron microscopy imaging and pre- versus postsynaptic phenotypic analysis. Below, we discuss the NMJ electrophysiological assay (see poster).

The electrophysiological recording assay for the third instar larval NMJ was described in the seventies (Jan and Jan, 1976), and a parallel assay based on patch clamping in embryos was developed later (Broadie and Bate, 1993). Briefly, the larva or embryo is filleted to expose the muscles along the body wall. A motor neuron is cut posterior to the ventral ganglion and drawn into an electrode in order to induce action potentials. A second electrode is placed in or on the muscle to measure the response. Both assays permit a very accurate characterization of a mutant's neuronal function but they are quite labor-intensive and thus are not the best tools to perform genetic screens. Instead, genetic screens for altered NMJ morphology have been performed to identify genes regulating synaptic bouton morphology (Aberle et al., 2002) because the reiterated pattern of NMJs along the body wall lends itself to rapid visual inspection. NMJ electrophysiological assays have allowed detailed characterization of numerous mutant genes whose human homologs were later shown to cause different human diseases. For example, *Shaker* (*Sh*) and *ether-a-go-go* (*eag*) mutations were isolated owing to the shaking phenotype of the legs (Kaplan and Trout, 1969). Subsequently, further characterization of these mutants using NMJ electrophysiological recordings revealed that the mutations affect potassium channel function (Jan et al., 1977; Wu et al., 1983). The vertebrate homologs of these potassium channels were later identified and shown to be involved in many human diseases, including cardiac arrhythmias, deafness and epilepsy (Jentsch, 2000). Another example is the *Drosophila* homolog of the human genes synaptotagmin 1 and 2, which was first shown in flies to be the calcium (Ca^{2+}) sensor for synaptic transmission, based on altered NMJ recordings (see poster) (Littleton et al., 1994). Recently, mutations in human synaptotagmin 2 have been discovered to cause Lambert-Eaton myasthenic syndrome, a rare autoimmune neuromuscular disorder (Herrmann et al., 2014).

Of note, the properties of *Drosophila* larval NMJs can be further studied using a variety of different assays, allowing an in-depth analysis that is not possible at any other synapse. These include live imaging of protein or organelle trafficking in the motor neuron axon or synapse (Andlauer and Sigrist, 2012), Ca^{2+} imaging of synaptic boutons (Macleod, 2012) and focal patch recordings from single boutons (Kurdyak et al., 1994). Given that fly NMJs are glutamatergic in nature, the similarities to mammalian CNS synapses provide us with a tool to study pathogenic mechanisms associated with neurological diseases, such as amyotrophic lateral sclerosis (ALS), spinal muscular atrophy and certain encephalopathies (Nahm et al., 2010; Pennetta et al., 2002; Sandoval et al., 2014; Sherwood et al., 2004; West et al., 2015). Collectively, these examples portray the instructive role of fly NMJ studies in identifying the pathogenic mechanisms of neuronal disorders.

Giant-fiber-system recordings

Although NMJ electrophysiological assays in flies are very useful to study synaptic development and transmission, they do not enable the analysis of neuronal function over time. Adult-specific assays are more appropriate to document gradual changes that occur in neuronal function with age, and are thus particularly useful for the study of neurodegenerative disorders. The giant fiber system (GFS) is one of the few neuronal circuits in adult flies that is amenable to electrophysiological recordings (Tanouye and Wyman, 1980). When flies detect a change in luminescence, the giant-fiber neurons in the brain signal to the thoracic ganglion to activate flight muscles [dorsal longitudinal muscles (DLMs)] and jump muscles [tergotrochanteral muscles (TTMs)] (King and Wyman, 1980). Electrophysiological recordings of the GFS can be made by stimulating the eyes while recording the depolarization in DLMs and TTMs (Tanouye and Wyman, 1980) (see poster).

The GFS assay has been extensively used to study features of epilepsy and seizures by high-frequency stimulation in the eye and detection of seizure-like electrical activity in the muscles. For example, gain-of-function mutations in the fly gene *paralytic* (*para*^{bss}) (Parker et al., 2011; Pavlidis and Tanouye, 1995) and loss-of-function alleles of *easily shocked* (Pavlidis et al., 1994) have a much lower threshold voltage of high-frequency stimulation to evoke seizure-like activity (Pavlidis and Tanouye, 1995). *para* encodes a voltage-gated sodium channel (Feng et al., 1995), and its human homologs are either associated with or shown to cause diseases such as encephalomyopathy, neuropathy and myoclonic epilepsy (Escayg et al., 2000; Meisler and Kearney, 2005). Recently, GFS assays have been used to document the demise of neurons in neurodegenerative studies and provide insight into underlying pathologies (Kerr et al., 2011; Lee et al., 2004; Watson et al., 2008; Dutta et al., 2016). For example, mutations in superoxide dismutase 1 (SOD1) in humans cause ALS (Rosen, 1993). Flies that overexpress wild-type human *SOD1* are unable to follow high-frequency stimulation in DLMs but exhibit a normal

Disease Models & Mechanisms (2016) 9, 235-244 doi:10.1242/dmm.023762

TTM response, indicative of interference of the GFS (see poster). Flies that overexpress human mutant *SOD1* have defective TTM and DLM responses, which worsen with age, consistent with age-dependent degeneration in ALS (Watson et al., 2008). This fly ALS model demonstrates the convenience of the GFS in neurodegeneration studies because it detects the progressive decline in motor function through electrophysiology.

Wing injury assay

The recently developed *Drosophila* wing injury assay is an elegant approach to study axonal degeneration and regeneration *in vivo* (Fang et al., 2012). The goal of these studies is to identify genes that are required for axonal degeneration and regeneration, and to identify the regulatory processes that are involved in spinal cord and nerve injuries. The fly peripheral nervous system has been intensively studied, which provides a great platform for this purpose. In the fly wing, mechanosensory and chemosensory neurons reside in the wing margin and project their axons toward the thoracic ganglion. Severing these axons using scissors or lasers causes degeneration of the distal portions of the axons (see poster) (Fang et al., 2012). After ∼7 days, the proximal portion of the injured axons regenerates by extending sprouts toward the lesion site. Alternatively, the axons regrow but invade another wing vein (Soares et al., 2014). These processes can be visualized by expressing a cytoplasmic green fluorescent protein (GFP) marker under the control of a neuronal *GAL4* driver. This simple assay is suitable for a large-scale forward genetic screen of viable as well as lethal mutations to identify genes that have not previously been implicated in degeneration or regeneration processes, because many of the genes and proteins required for these processes are evolutionarily conserved. By using this assay, a forward genetic screen identified mutations in *highwire*, a RING domain E3 ubiquitin ligase, that suppresses the degeneration of the distal portion of the axons upon axotomy (Neukomm et al., 2014). Furthermore, another screen based on this wing injury assay led to the discovery that downregulation of JNK signaling promotes axon regeneration (Soares et al., 2014). In summary, this novel technique capitalizes on the well-characterized fly peripheral nervous system and can provide clues about the molecular mechanisms that underlie the degeneration and regeneration of neurons.

Cardiovascular assays

Fly models of cardiovascular diseases first emerged 20 years ago together with the development of assays to measure heart development and function (Bodmer, 1993; Ocorr et al., 2014). The *Drosophila* heart, called the dorsal vessel, differs from the human heart in that it is an open circulatory system consisting of a hollow, muscular tube closed at the posterior end. The vessel runs longitudinally from the posterior abdomen (heart proper) into the thorax (aorta). Similar to the human heart, which consists of distinct chambers, the fly heart is also divided into four chambers that are separated by small valve-like openings through which blood, or rather the analogous fluid in insects, hemolymph, enters the heart (Lehmacher et al., 2012). Each chamber consists of six myocardial cells to facilitate the flow of hemolymph through the dorsal vessel. The aorta, which is made up of myocardial cells that do not contract very much, is a tube that facilitates the transport of hemolymph to the head, from where it flows into the body cavity (Bier and Bodmer, 2004). The molecular pathways underlying the development of the fly heart and its function have provided valuable information relevant to human heart development and physiology. For example, *tinman* (*Nkx2-5* in humans), a homeobox transcription factor identified in flies, is required for heart development (Bodmer, 1993). Mutations in the human homolog of this gene were later shown to cause congenital heart disease and have subsequently been shown to cause sudden cardiac arrest in middle age (Schott et al., 1998). Moreover, the discovery of *pannier* (GATA4) and *neuromancer* (Tbx20) transcription factors revealed a conserved cardiogenic network, which enabled the study of these factors in human heart development and function (reviewed in Qian and Bodmer, 2012). The fly heart proves to be a convenient invertebrate heart disease model owing to conserved molecular pathways and the variety of assays to study different aspects of heart disease. In the following section, we will discuss a few of the cardiovascular assays and how they provide mechanistic insight about human heart disease. We refer readers to excellent reviews on different aspects of the topic for further information (Diop and Bodmer, 2015; Choma et al., 2011; Ocorr et al., 2014; Wolf and Rockman, 2011).

Heartbeat measurement

Similar to mammals, the fly heartbeat consists of a cardiac cycle that includes diastolic and systolic periods. Interestingly, the cardiac cycle in adult flies is composed of alternating anterograde and retrograde beats (Wasserthal, 2007), leading to a periodic change in the flow of hemolymph (Dulcis and Levine, 2005). Measuring the heartbeat rate and rhythmicity is one of the fundamental assays to determine heart function. Measurements of the heartbeat, either in the dissected dorsal vessel of the larva or the adult abdomen, are based on visual recordings. These methods rely on the optical intensity of light passing through the heart while it beats (Gu and Singh, 1995). The approaches are relatively fast and can be used to assess the function of individual genes while also being appropriate for forward genetic screens.

Nowadays, further insight into cardiac function can be obtained by combining the standard heartbeat measurement assay with the use of a high-resolution camera and computer algorithms to detect optical intensity changes. This improvement of the method, semi-automated optical heartbeat analysis (SOHA), allows simultaneous assessment of systole, diastole and rhythmicity (Fink et al., 2009; Ocorr et al., 2009). This assay has been instructive in the study of various disease models, including channelopathies (Ocorr et al., 2007b), cardiomyopathies, age-dependent heart defects (Gill et al., 2015) and heart dysfunction associated with non-cardiac conditions, such as myotonic dystrophy (Chakraborty et al., 2015). Young wild-type flies monitored with SOHA show rhythmic cardiac contraction that gives rise to a characteristic M (motion)-mode trace. As these animals age, the contraction becomes arrhythmic (Ocorr et al., 2007a), analogous to cardiac arrhythmias observed in elderly humans (Jones, 2006). Apart from aging, the genetic cardiac fly models also recapitulate human cardiac disorders and allow in-depth analysis of genes involved in cardiovascular disease. For instance, dominant mutations in the human gene alpha-B crystallin ($CryAB^{R120G}$), which encodes a chaperone, cause defects that underlie a range of diseases, including cardiomyopathies. Similarly, overexpression of the human $CryAB^{R120G}$ mutant transgene in the fly results in dilation of the dorsal vessel (see poster), mimicking dilated cardiomyopathy in humans (Xie et al., 2013).

Optical coherence tomography (OCT)

The heartbeat assays described above all require the dissection of a semi-intact fly heart. More sophisticated tools such as optical coherence tomography (OCT), the equivalent of echocardiography in humans, permit the non-invasive characterization of the

Disease Models & Mechanisms (2016) 9, 235-244 doi:10.1242/dmm.023762

Drosophila heartbeat *in vivo* (Wolf et al., 2006). OCT uses laser beams to scan the entire tissue and subsequently uses the scattered light to produce subsurface images (see poster). This technique allows imaging of the heart in awake adult flies. This method is also convenient to produce M-mode images that reveal subtle changes in cardiac movement, similar to other video-based techniques. Yet, it should be kept in mind that, because this method requires downstream processing, it is relatively slow and is not very convenient to measure heart rhythmicity. Although it is costly and specialized, once established, it can be used for screening, however. Indeed, a deletion screen designed to identify cardiomyopathy genes via OCT led to the discovery that a component of Notch signaling, *weary (wry)*, could play a role in dilated cardiomyopathy (Kim et al., 2010). The protein encoded by *wry* has non-canonical Notch motifs, and overexpression of Serrate, a ligand of Notch, can rescue the cardiomyopathy in *wry* mutants. The results of this study indicate a potential role of Notch signaling in the adult heart and suggest that it can be a therapeutic target for dilated cardiomyopathies. In humans, Notch signaling is important for the organogenesis of the heart (de la Pompa and Epstein, 2012) and mutations in NOTCH1 cause aortic valve disease (Garg et al., 2005). Although the importance of Notch signaling in mammalian heart development has been studied, its role in the adult heart, especially in a regenerative context, is an emerging area that has many unexplored questions (Ferrari and Rizzo, 2014).

Ca^{2+} measurement

Normal cardiac physiology requires controlled Ca^{2+} handling for proper contraction of cardiomyocytes, in flies as well as in humans. Defects in cardiac Ca^{2+} homeostasis are observed in cardiomyopathies and heart failures (Guo et al., 2006). Intracellular Ca^{2+} levels can be quantified using genetically encoded Ca^{2+} indicators (GECIs), such as GCaMP (a fusion protein of green fluorescent protein, calmodulin and a peptide sequence from myosin light chain kinase). These Ca^{2+} sensors have been successfully used to assess cardiac Ca^{2+} pulses by expressing them specifically in the fly heart (see poster) (Lin et al., 2011). Dilated cardiomyopathies are associated with altered Ca^{2+} homeostasis. For example, *held-up* mutant flies (Hdp^{2}) contain a point mutation in troponin, a protein required for proper cardiac muscle contraction, and have enlarged diastolic chambers (Wolf et al., 2006). hdp^{2} mutants display a prolonged duration of the Ca^{2+} peak intensity (see poster), indicating that the late cytosolic Ca^{2+} rise is delayed. This delay, along with protracted Ca^{2+} indicator fluorescence, is speculated to be the result of impaired Ca^{2+} reuptake into the sarcoplasmic reticulum (Lin et al., 2011). Moreover, Ca^{2+} measurements are also useful in the study of age-related cardiac defects, because aging flies display a decrease in the maximal rate of Ca^{2+} fluorescence decay, indicating that spontaneous cardiac frequency is reduced (Santalla et al., 2014). Older flies have variable spontaneous cardiac frequencies, which indicates an arrhythmia in the aging heart. As mentioned previously, elderly humans also demonstrate cardiac arrhythmias. Hence, the result of this study proposes that alterations in Ca^{2+} signaling might be related to arrhythmias observed in the elderly.

Field potential and intracellular recording

Heart rhythmicity is maintained through electrical conduction. The assays described above to monitor fly heart rhythm do not directly measure electric conduction. For this purpose, electrophysiological methods have been developed to record heart field potential directly, and these approaches can be applied in both larval and adult fly hearts (Papaefthimiou and Theophilidis, 2001). Spontaneous heart field potentials can be measured via a thin glass electrode, which is placed in contact with the semi-intact fly heart (see poster) (Cooper et al., 2009). In larvae, the heart is loosely attached to the body wall, and a floating electrode technique can therefore be used to minimize damage (Lalevée et al., 2006). By contrast, the adult heart adheres more tightly to the body wall, and hence recordings are performed from the myocardium of the heart chamber (Dulcis and Levine, 2005) by stimulating a local glutamatergic input. The action potential recorded from both the larval and adult hearts display a pacemaker potential that is a feature of the myogenic heart. Although these electrophysiological methods are not very convenient for high-throughput screening, they are particularly useful to understand the mechanistic properties of the heart. For example, *KCNQ1* (a gene that encodes a voltage-gated potassium channel) mutant flies display cardiac arrhythmia that worsens with age (Ocorr et al., 2007b). KCNQ1 is partially responsible for the rhythmic contraction of the heart muscle. The electrophysiological recordings from the *KCNQ1* mutant fly hearts show reduced repolarization ability, most probably due to a decrease in the repolarizing K^{+} current (see poster). Interestingly, in humans, mutations in KCNQ1 cause many cardiac disorders, such as familial atrial fibrillation, which is characterized by uncoordinated cardiac electrical activity, and long-QT syndrome, which is also characterized by rapid heartbeats (Bellocq et al., 2004; Johnson et al., 2008). These studies collectively support the use of *Drosophila* as an effective model to study heart disease.

Oenocyte and fat body assays

Liver disease causes millions of deaths per year worldwide (Byass, 2014). Nonalcoholic fatty liver disease (NAFLD) is the most common form of liver disease, affecting 75-million to 100-million individuals in the USA (Rinella and Sanyal, 2015). Because the burden of this disease is large and costly, the elucidation of pathogenic mechanisms underlying liver disease, using model organisms, is a key healthcare priority. In humans, the liver has many metabolic functions, including detoxification of metabolites, protein synthesis, synthesis of digestive metabolites and maintenance of blood glucose levels. These functions are performed by highly specialized cells named hepatocytes. To regulate fat usage during starvation, adipocytes – the body's major fat-storing cells – break down lipids into fatty acids (FAs) via adipocyte triglyceride lipase (ATGL) (Zechner et al., 2005). The FAs are secreted in the bloodstream, taken up by the liver and processed via hepatocytes. During prolonged starvation, hepatocytes synthesize water-soluble ketone bodies from the FAs, and these are released into the bloodstream to be used as an energy source for other tissues (Green et al., 2015).

Fasting fly larvae similarly release lipids from the fat body – the organ responsible for energy storage and utilization – and these lipids are taken up by specialized cells named oenocytes (Chatterjee et al., 2014). Until recently, the fly fat body was thought to be the functional homolog of the human liver (Baker and Thummel, 2007). However, studies have shown that fly oenocytes are more similar to hepatocytes than is the fat body, based on their response to starvation. Furthermore, oenocytes express 22 homologs of human fat-metabolizing genes expressed in hepatocytes, and also express genes involved in hepatocyte differentiation, including *hepatocyte nuclear factor 4-a* (*Hnf4-a*) and *COUP-transcription factor* (*COUP-TF*) (Gutierrez et al., 2007). Finally, oenocyte-specific knockdown of acetyl-coenzyme A-carboxylase (ACC), a rate-limiting enzyme in FA synthesis, results in lethality, demonstrating the importance of oenocytes for FA synthesis (Parvy et al., 2012).

Disease Models & Mechanisms (2016) 9, 235-244 doi:10.1242/dmm.023762

Overall, recent data demonstrate that the fat body and oenocytes in flies are the functional homologs of the vertebrate liver (see poster). In the next section, we will review the fundamental fat body and oenocyte assays to model liver diseases in flies. *Drosophila* has, in our opinion, not been exploited to its full potential as a model system in liver disease research, and there is scope for the development of new assays and the improvement of existing ones.

Lipid-droplet accumulation

The assays that are used to study fat body and oenocyte function in flies typically depend on visualizing lipid storage in response to differential nutritional conditions. Generally, dyes such as Oil Red-O (Gutierrez et al., 2007) or BODIPY (Kohyama-Koganeya et al., 2008) are used to visualize lipids in oenocytes and the fat body of the fly. An *in vivo* assay to determine the presence of lipids is based on stimulated Raman scattering (SRS) microscopy or coherent anti-Stokes Raman scattering (CARS) microscopy (Chien et al., 2012). These spectroscopic assays detect the vibrational signature of molecules and allows labeling-free, live imaging of lipids (see poster).

Mutations in human HNF4A are associated with a type of inherited diabetes known as maturity-onset diabetes of the young, type 1 (MODY1) (Yamagata et al., 1996). These mutations lead to decreased serum triglyceride (TAG) levels, and the onset of diabetes, in affected individuals (Fajans et al., 2001). The functional homolog of *HNF4A* in flies is *Hnf4*. *Hnf4*-null flies are very sensitive to starvation because they are unable to harness energy from stored lipids (see poster) (Palanker et al., 2009). Interestingly, many enzymes involved in lipid catabolism are upregulated in starved *Hnf4*-null animals. Investigation of these mutant flies has provided supporting evidence that a specific FA activates the nuclear hormone receptor, which in turn stimulates energy production by activating FA oxidation.

In a forward genetics study, a genome-wide RNAi screen performed in fly oenocytes identified multiple obesity-related genes that are also associated with obesity in mice (Pospisilik et al., 2010). One of the candidates from this screen is the fly homolog of fatty-acid elongase (*ELOVL6*), *baldspot*. Interestingly, mouse mutants of *Elovl6* also develop obesity and hepatosteosis (Matsuzaka et al., 2007). To date, mutations in human *ELOVL6* have not been linked with obesity or liver disease, however. Another obesity model in flies is the fat-body-specific overexpression of *Lipid storage droplet 2* (*Lsd-2*) (Grönke et al., 2003). These flies are obese, resistant to starvation and have elevated TAG storage. Furthermore, CARS microscopy revealed that starved flies that overexpress *Lsd-2* in the fat body have reduced lipids in oenocytes, showing that they are unable to store lipids in oenocytes as a starvation response (see poster) (Chien et al., 2012). The functional homolog of *Lsd-2* in humans is perilipin 2 (*Plin2*) (Rajan and Perrimon, 2013). Perilipins coat intracellular lipid droplets and are involved in lipolysis. Interestingly, the liver biopsies from nonalcoholic steatohepatitis patients show increased levels of PLIN2 in lipid droplets, indicating the importance of *Plin2* for individuals with NAFLD (Fujii et al., 2009). In addition to genetic risk factors, environmental factors that result in liver disease can also be studied in flies. Obesity and type 2 diabetes are the major risk factors for NAFLD (Neuschwander-Tetri et al., 2010). Fly larvae that are fed with a high-sugar diet mimic the hallmarks of both obesity and type 2 diabetes (Owusu-Ansah and Perrimon, 2014). The high-sugar-fed larvae have increased body fat along with the accumulation of large lipid droplets in the fat bodies (Musselman et al., 2011). The transcriptional profile of these larvae demonstrates an increase in the expression of genes related to lipid catabolism. Of note, the transcriptional profile of these larvae is similar to *Hnf4*-null flies, indicating a conserved transcriptional signature that might be instrumental in diabetes and liver disease. In summary, these results provide compelling evidence that the fly can help provide a valuable mechanistic understanding of processes that cause liver diseases.

Nephrocyte and Malpighian tubule assays

The function of the human excretory system is to eliminate metabolic waste and maintain a homeostatic ion balance. The nephron is the basic structural and functional unit of the human kidney. It is composed of a glomerulus, glomerular capsule and renal tube. The glomerular podocyte is an epithelial cell that wraps around capillaries in the glomerulus and plays an important role in the filtration of blood to produce urine. Glomerular podocytes create the filtration barrier by sending out interdigitating processes that are separated by 30- to 50-nm-wide slit pores, called the slit diaphragm (see poster) (Wartiovaara et al., 2004). Blood is filtered through these slit diaphragms, and mutations in several genes that disrupt the filtration barrier lead to kidney failure (Kestilä et al., 1998; Patrakka et al., 2000). Although invertebrates lack nephrons, *Drosophila* nephrocytes share remarkable similarity to podocytes. There are two different nephrocytes in *Drosophila*: the pericardial nephrocytes, which flank the fly aorta, and garland nephrocytes, which form a ring around the proventriculus (Denholm et al., 2013). These nephrocytes exhibit extensive folds of the plasma membrane and create ∼30-nm slit pores (Weavers et al., 2009), forming the nephrocyte diaphragm, which shares functional and molecular similarities with the slit diaphragm of the mammalian podocyte (see poster). Indeed, similar to the mammalian podocyte, the proteins encoded by *sticks and stones* (*sns*) and *dumbfounded* (*duf*), the *Drosophila* homologs of nephrosis 1 (*NPHS1*) and nephrin 1 (*NEPH1*), form homo- and heterotypic interactions across the slit pore of nephrocytes in flies (Weavers et al., 2009).

Besides nephrocytes, the fly uses Malpighian tubules to clear toxins, produce uric acid, regulate ions and acid-bases, and balance fluid (Beyenbach et al., 2010). Again, many genes, such as the vacuolar-type-ATPases (V-ATPases), Na^+/K^+-ATPase, aquaporins and several ion channels and transporters, are shared between flies and mammals and are involved in ion homeostasis (Wang et al., 2004). Interestingly, mutations in the *rosy* (*ry*) and *maroon-like* genes in fly lead to a sensitivity to dietary purines and bloated, malformed Malpighian tubules (Hadorn and Schwinck, 1956). *ry* encodes a xanthine oxidase, and its deficiency in humans causes type I xanthinuria (an inborn error of metabolism) (Dent and Philpot, 1954). Biochemical studies of the fly mutants have provided a clear understanding of the source of the disease: accumulation of xanthine, the *ry* enzyme's substrate (Bonse, 1967; Glassman and Mitchell, 1959; Kamleh et al., 2008; Mitchell and Glassman, 1959). In addition, recently, Malpighian tubules have been used to model kidney-stone formation. Because the tubules are transparent, they enable observation of stone nucleation and growth of oxalate crystals in flies (Hirata et al., 2012; Landry et al., 2015).

Nephrocyte filtration assay

The nephrocyte filtration assay is based on the nephrocyte's ability to take up fluorescently labeled dextrans with different molecular masses: nephrocytes filter the hemolymph with a size-dependent efficiency (see poster) (Weavers et al., 2009). By using this assay as well as other approaches, two key genes were identified: *sns* and *duf*. Mutations in human homologs of *sns* cause congenital nephrotic syndrome (Kestilä et al., 1998), whereas loss of the *duf* homolog in mice disrupts the slit diaphragm and causes nephrotic syndrome at birth (Donoviel et al., 2001; Liu et al., 2003).

Disease Models & Mechanisms (2016) 9, 235-244 doi:10.1242/dmm.023762

The nephrocyte filtration assay is a convenient assay to identify new genes in forward genetic screens. Indeed, an RNAi screen based on a modified filtration assay identified many genes that are required for nephrocyte function (Zhang et al., 2013a). Instead of dextrans, the authors overexpressed a secreted fluorescent peptide, rat atrial natriuretic factor-GFP (ANF-GFP) from muscle and observed the uptake of the peptide in pericardial nephrocytes (see poster). Several genes that are required for nephrocyte filtration function were identified via this preliminary screen. The human homologs of these genes are linked to renal diseases, including *mec2*, *CG11592* (the *Drosophila* homolog of mammalian amnionless) and *CG32702* (the *Drosophila* homolog of mammalian cubilin) (Boute et al., 2000; Storm et al., 2011; Wahlstedt-Froberg et al., 2003).

The function of Malpighian tubules can be determined by visually assessing their transparency (not shown in poster). For example, loss-of-function mutations in the V-ATPase (*vha55*), a transmembrane protein required for proton transport, was identified via this assay in *Drosophila* (Davies et al., 1996). It was then observed that every mutation in a gene that encodes subunits of the V-ATPase complex leads to a transparent Malpighian tubule, indicating a defect in acidification (Allan et al., 2005; Davies et al., 1996; Dow, 1999). Later, a mutation in the human B_1 subunit of the V-ATPase was discovered to cause renal tubular acidosis (Karet et al., 1999). In summary, the *Drosophila* nephrocytes and Malpighian tubules have been shown to be valuable models in the study of human renal diseases.

Concluding remarks

Drosophila provide a powerful platform to perform functional annotations of human genes and disease variants, given the observation that evolutionarily conserved genes tend to have similar molecular functions. The fly community is continually providing state-of-the-art tools and resources that are rapidly evolving and permit efficient gene and genome engineering. The assays described here permit evaluation of how these genes affect specific cellular processes and allow us to study the molecular mechanisms that underlie diseases of the nervous, cardiovascular, metabolic and renal systems, and beyond. Furthermore, the assays that can be used in genetic screens should allow us to uncover as-yet-uncharacterized disease-causing genes.

This article is part of a subject collection on Spotlight on *Drosophila*: Translational Impact. See related articles in this collection at http://dmm.biologists.org/collection/drosophila-disease-model.

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Competing interests

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