Drosophila as a Model for Human Diseases

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The fruit fly has long been a powerful organism for high-throughput *in vivo* studies in biology and genetics. Studies in *Drosophila* offer a simple platform, modelling multiple human diseases. With an ever-expanding genetic toolkit, the fly genome has become increasingly accessible to experimental manipulation. The ectopic expression of human disease genes involved in neurodegenerative and neurodevelopmental disorders have elucidated genetic modifiers that have been revealed to also cause human disease or to be potential drug targets for human neurological disorders. Recently, fly models are being used to study other human pathology such as cancer, infections and cardiovascular disorders. Lastly, 'humanisation' strategies to replace endogenous fly genes with their human homologue have begun. This has been particularly beneficial in assessing putative pathogenic variants implicated in rare Mendelian disorders, and thereby aiding diagnosis. In summary, the fly has remained a relevant platform for modelling human disease and elucidating genetic pathways and potential therapeutic targets.

Introduction

Drosophila melanogaster, the fruit fly, has been indispensable in furthering our understanding of molecular genetics and biology. The short generation time of flies and the ease of culturing them in vast numbers, combined with a myriad of steadily advancing genetic tools make 'the vinegar fly' an economical platform to study a wide variety of biological process. Early work in the twentieth century by Thomas Hunt Morgan experimentally confirmed the hypothesis that genetic information was carried on chromosomes. In the decades since, unbiased screens and genetic interaction studies in flies has helped elucidate entire signalling cascades that are tightly conserved across the tree of life (Johnston, 2002). More recently, *Drosophila* research has expanded to aid in understanding and modelling human disease. While the fly shares approximately 55–60% of homologous genes with humans, there is an enrichment of disease genes such that 75% of known disease-causing genes are conserved in the fly (Bier, 2005). Moreover, in unbiased forward genetic screens for lethal mutations in *Drosophila,* a significant (93%) number of genes are conserved in humans with 30–45% annotated in the OMIM (Online Mendelian Inheritance in Man) indicating a potential resource to elucidate other essential genes possibly connected to disease (Yamamoto *et al*., 2014). In fact, comparative analysis utilising the fly expression database, FlyAtlas, reveals that there is common selective expression of human disease genes and their respective fly homologues in analogous tissues (Chintapalli *et al*., 2007). *Drosophila* offer an added simplicity as one fly gene typically shares homology with multiple human paralogues as a result of duplication events over the course of vertebrate evolution. This lack of genetic redundancy in flies can be used to tease out what human paralogues are functionally conserved across species and which genes have either lost common functions or have become more specialised. This genetic redundancy can help create robust phenotypes in loss-of-function studies as the fly lacks many of the compensatory paralogues of its vertebrate cousins. It is important to note that while fly models of human disease may not directly mimic human symptomatology, many cellular, molecular and genetic studies can be performed to offer insight. This is true for biologically conserved pathways and also remarkably in cases where homology of the disease-causing gene is lacking. This type of analysis requires one to look past trying to 'model' a human disease in a fly and instead to use the fly to gain insight into genetic mechanisms of disease. In this article, we will briefly summarise the key advances of both the *Drosophila* genetic toolkit and current human disease models in the field

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focusing on six major disease categories including neurodegenerative/neurodevelopmental disorders, cancer, cardiac and muscle, the response to infection and Mendelian disease. Finally, we describe recent 'humanisation' strategies to functionally validate putative variants in human disease.

Drosophila Engineering Tools for Human Disease

Dramatic advances in human genome sequencing and clinical application of sequencing has created vast repositories of human genomic variation. Determining how this vast expanse of genotype variation in humans contributes to health and disease is the subject of enormous efforts in bioinformatics, genomics, cell-based studies and model organism research. With this rapid expansion in our knowledge of human genotype variation, there have been concurrent advances in the genetic manipulation of *Drosophila* to disrupt genes, control gene expression and perform gene replacement *in vivo*. The combination of the great need for studying human genes and the toolkit in *Drosophila* have brought flies into the forefront of model organisms in research of genetic disease. Any gene is now open to mutation, modulation and tagging (**Figure 1**).

Techniques for fly gene loss of function

The cornerstone of genetic manipulation in flies has been unbiased forward genetic screens, most typically performed by ethyl methanesulfonate (EMS)-induced mutagenesis (**Figure 1a**). Many classical null and hypomorphic alleles have been created in this way. Secondly, transposable elements can be inserted across the genome to act as a mutagen, enhance transcription and fluorescently label endogenous proteins. The most well-known of these have been *P*-elements, *piggyBac*-elements and Minos insertions (Bellen *et al*., 2011) (**Figure 1b**). Recently, using recombination-mediated cassette exchange (RMCE) using ΦC31 integrase, transgenes may be swapped to offer an increasing amount of manipulation (Diao *et al*., 2015; Venken *et al*., 2011). While transposable elements offer a wide range of manipulation, they do succumb to nonrandom insertion into the genome and therefore prevent exhaustive genome-wide gene disruption capabilities. However, this has largely been overcome by the development of large collections of elements with different site preferences (Bellen *et al*., 2011).

Exogenous expression of transgenes or gene knockdown

The UAS (upstream activation sequence)-GAL4 system remains the cornerstone of conditionally expressing or downregulating (via RNAi (ribonucleic acid interference)) genes. This is a binary system that provides both spatial and temporal control of gene expression. Briefly, a fly containing the yeast transcriptional activator GAL4 under control of a desired promoter (also called driver line) is crossed to a fly containing the transgene (cDNA (complementary deoxyribonucleic acid), RNAi, enhanced green fluorescent protein (eGFP), etc.) downstream of an UAS, whereas GAL4 binds and promotes transcription of the desired transgene (**Figure 1c** and **d**). Analogous bipartite systems have also been developed such as the LexA/LexAop and QF/QUAS systems in which the general theme of strains providing conditional expression can be combined with strains providing a transgene under control of that system. The availability of multiple binary systems allows complex control of multiple genes. For example, different genes can be expressed in different tissues of the fly by combining the UAS-GAL4 system with another system. Further adaptations to these systems have also provided inducible methods, more specific tissue refinement in conjunction with split GAL4, and repressible mechanisms using GAL80 or QS components (Southall *et al*., 2008). The deGradFP system allows endogenously tagged proteins to be quickly depleted at the protein level permitting minimal off-targets and spatial and temporal control of protein expression (Caussinus *et al*., 2013). In sum, conditional expression of any gene in virtually any cell or tissue type at any life stage of the organism is at the fingertips of the fly biologist.

Mosaic analysis in flies

Another key tool widely used in *Drosophila* is developing animals that have both wild-type and identifiably mutant cells. This is particularly useful to examine the effect of germline-lethal mutations in a defined tissue or tissues. Mitotic clones may be generated by expressing flippase (FLP) recombinase under a heat shock promoter to initiate site-specific recombination of FLP recombinase target (FRT) sites integrated into the *Drosophila* genome (Johnston, 2002). FRT chromosome may be marked with GFP, which allows homozygous mutant clones that lack GFP to be identified. Mosaic analysis with a repressible cell marker (MARCM) has been a powerful advance that uses the heat shock FLP/FRT system. MARCM allows mutant cells to be identified by a marker such as GFP, whereas wild-type cells remain unlabelled (**Figure 1e**). Combined with the tools already discussed, MARCM provides another way to examine cellular effects from transgene expression or lineage tracing within mutant clones (del Valle Rodríguez *et al*., 2012).

CRISPR gene editing in flies

The most recent and popular area of research has been the development of novel tools utilising the *Clustered regularly interspaced short palindromic repeats* (CRISPR)-Cas9 system. The Cas9 endonuclease is utilised to cleave chromosomes at user-defined sites dictated by specific guide ribonucleic acid (gRNA) and taking advantage of intrinsic homology-directed repair (HDR) mechanisms. This system offers the ability to edit single nucleotides or to insert a desired construct conceivable anywhere in the genome. Genes may be quickly targeted and knocked out by insertion of a construct containing a gRNA targeting a gene with Cas9 and appropriate homology arms that flank the genomic sequence of the desired insertion (**Figure 1f**). HDR will occur and the Cas9 will then target the second allele, leading to homozygous mutants in the first generation.

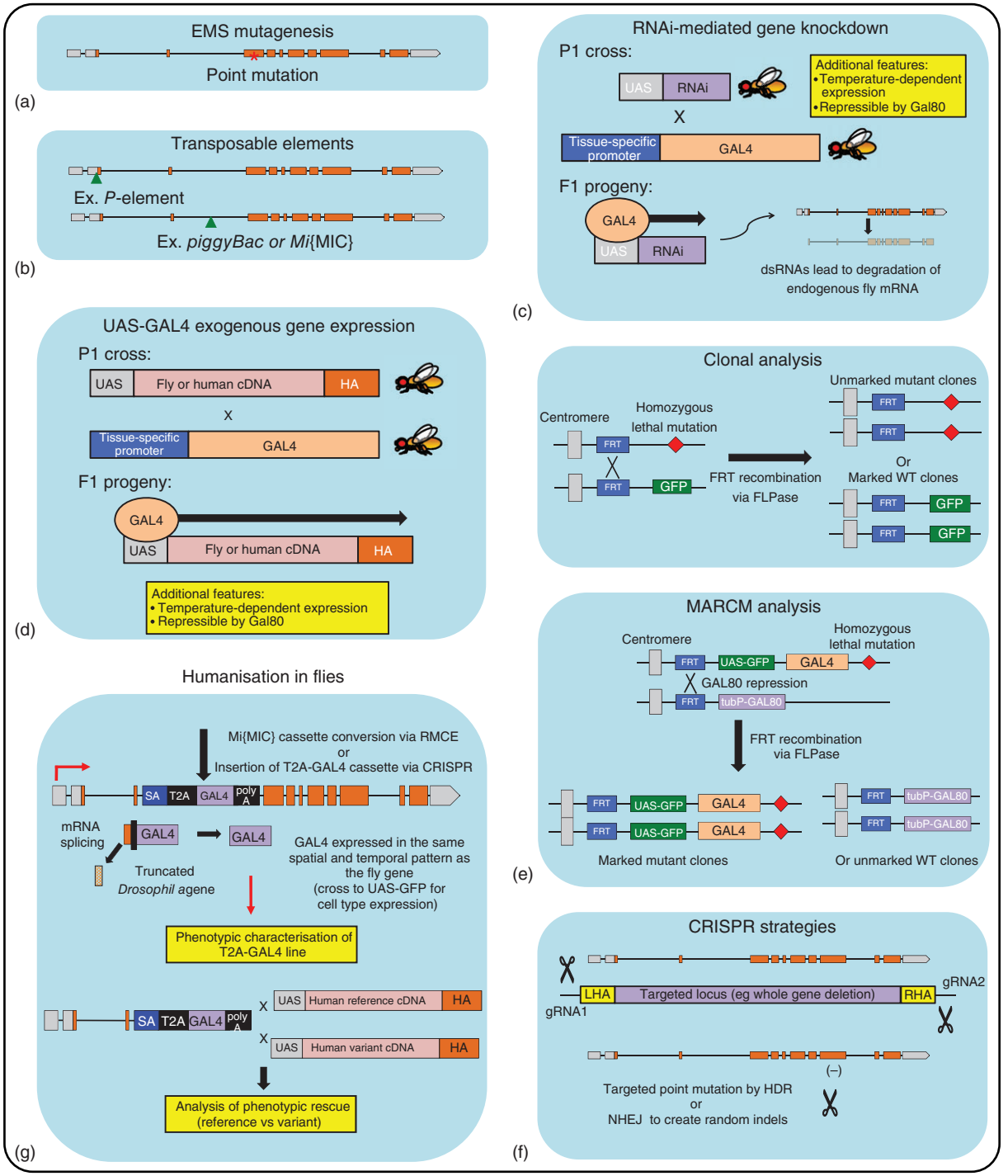

Figure 1 **Highlights of the *Drosophila* genetic toolkit.** (a) Fly genes have classically been randomly disrupted by mutagens such as EMS (ethyl methanesulfonate). The allele depicted in shows a point mutation indicated by the red asterisks in an exon (orange boxes) of a fly gene. (b) The use of transposable elements (green triangles) such as *P*-elements has allowed for gene disruption with a variety of cassettes but is limited by insertional 'hot-spots'. (c) GAL4 binding the UAS under control of a tissue-specific driver may be used to knock down fly genes using RNAi (ribonucleic acid interference). (d) Overexpression of constructs such as cDNA (complementary deoxyribonucleic acid) (i.e. fly, mouse or human) or fluorescent reporters in a tissue-specific manner. (e) Generation of mitotic clones via FRT recombination has allowed for biological study of essential genes that typically cause lethality by examining homozygous mutant cells in a mostly wild-type tissue. (f) CRISPR strategies have allowed targeted gene disruption to either make gene knockouts/knock-in animals or insert any user-defined cassettes. (g) Intronic insertion between coding exons of a T2A-GAL4 cassette via CRISPR or MiMIC cassette conversion by RMCE (recombination-mediated cassette exchange) allows for fly gene loss of function and subsequent 'humanisation' and replacement with human cDNA to test for conserved function and potential disease-associated variants.

This technique, entitled 'gene drive', is effective for rapid F_1 screening and has wide implications for controlling disease and parasites in the wild (Gantz *et al*., 2015). Multiple groups have shown that the CRISPR-Cas9 system is a highly efficient strategy that can be used to 'knockout' or 'knock-in' heritable mutations and manipulate genes previously unexploited by transposable elements (Fillip Port, 2016). The full potential of harnessing all of these tools available has yet to be pursued in studying human disease.

Humanisation in flies

One of the newest tools and concepts in the field is 'humanisation' of the fly (Bellen and Yamamoto, 2015; Chow and Reiter, 2017). This process starts with replacement of the endogenous fly gene with a T2A-GAL4. This can be accomplished by replacing an existing coding MiMIC line (a line with a MiMIC cassette inserted between two coding exons) with an exogenous DNA fragment containing a T2A-GAL4 via RMCE (Diao *et al*., 2015). This causes a truncation of the fly gene and transcription of a GAL4 (**Figure 1g**). These flies can then be characterised for loss-of-function phenotypes (lethality, lifespan, behaviour, etc.) and we can then 'humanise' the fly by crossing to UAS-human cDNA thereby providing the fly with the human protein replacing the loss of the fly protein. Provided the human cDNA can rescue the phenotype, any putative variants implicated in disease can be then tested for functional outcomes. This strategy will be explored further in the section concerning Mendelian disorders and rare or undiagnosed diseases.

Drosophila as a Model for Human Disease

Given the plethora of genetic tools available described earlier, *Drosophila* genes can be extensively manipulated to model human disease. Genes implicated in familial forms of disease can be used to model a wide variety of phenotypes including neurological, oncogenic, cardiac and immune. In the following sections, we present how flies have furthered our knowledge in these disorders and are summarised in **Table 1**. **See also**: ***Drosophila* as a Model for Human Diseases**

Neurodegenerative diseases

Neurodegenerative disorders encompass a wide range of diseases that involve a dysfunction of the maintenance of neurons over the life of the organism. Across many of these disorders, similar pathological features can be noted such as accumulation of misfolded proteins and mitochondrial dysfunction. The fly offers the ability to model the human disease in terms of both neurological/behavioural and cellular/molecular phenotypes. Some of the most common assays include survival, learning and memory function, locomotor activity (both in larvae and adult), bang sensitivity, flight, brain vacuolisation, retinal degeneration, transduction studies using electroretinogram (ERG) or neuromuscular junction (NMJ) recordings as well as any cellular and molecular changes (**Figure 2**). The field has benefited from large-scale screens, typically modelling easily scorable phenotypes in the eye or wing of the fly caused by the ectopic expression of a disease-linked gene (Fernandez-Funez *et al*., 2000; Marcogliese

Table 1 Common assays and genes modelled in flies related to human disease.

Human disease	Associated genes/pathways	Common fly assays	Key reference
Neurodegenerative disorders			
Alzheimer's disease	*APP, MAPT*	Eye and wing morphology[a], learning and memory	Lenz *et al.* (2013)
Parkinson's disease	*SNCA, LRRK2, PRKN, PINK1, PARK7*	Eye and wing morphology[a], climbing and activity, dopamine neuronal loss, mitochondrial	Lu and Vogel (2009)
Huntington's disease	*HTT*	Eye and wing morphology[a]	McGurk *et al.* (2015)
Spinocerebellar ataxia 3	*ATAXIN3*	Eye and wing morphology[a]	McGurk *et al.* (2015)
Neurodevelopmental disorders			
Rett syndrome	*MECP2*	Eye and wing morphology[a]	Cukier *et al.* (2008)
Fragile X syndrome	*FMR1*	Neuronal morphology	Coffee *et al.* (2010)
Angelman syndrome	*UBE3A*	Climbing, abnormal circadian rhythms, learning and memory	Wu *et al.* (2008)
Cancer	*Ras, hippo, Myc*	Clonal analysis, border cell migration	de la Cova *et al.* (2004); Vidal and Cagan (2006)
Cardiovascular disorders	*MEF2A, HAND2, miR1*	Heart-/muscle-specific gene ablation optical coherence tomography	Nishimura *et al.* (2011)
Inflammation and infectious disease	TLRs, NFkB	Bacterial infection microbiome studies	Lemaitre and Hoffmann (2007); Fischer *et al.* (2017)

[a]Typically performed via ectopic expression of human disease gene.

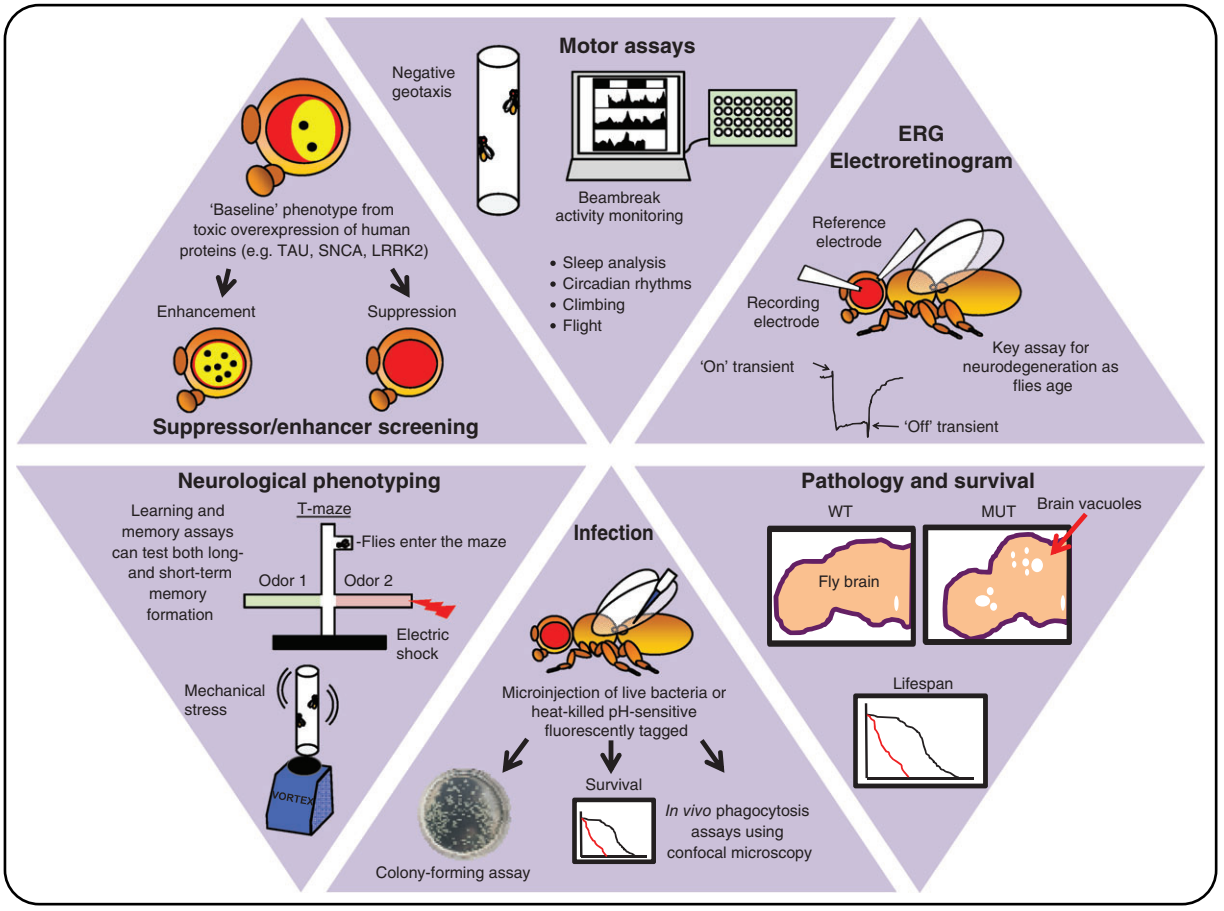

Figure 2 Assays for phenotypic characterisation of *Drosophila* in models of human disease. The diagrams depict only some of the common assays involved in using *Drosophila* to study human disease. Triangles from the left and clockwise: (1) Suppressor/enhancer screening has been a consistent method for revealing genetic modifiers of toxic gene expression, which has played a predominant role in neurodegenerative disorders. (2) Many neurological disorders present with motor dysfunction and flies can be analysed for climbing or flight defects. In addition, activity monitors that use an infrared beambreak system can be an automated method to measure activity, sleep and circadian rhythms. Not depicted are fly tracking software that can measure a variety movement phenotypes on top of other behaviours after video capture. (3) Electrophysiological recordings by electroretinogram are an easy method for modelling neurotransmission defects in flies. Represented is a stereotypical response after stimulation that can be compared to mutant flies. (4) Particularly in diseases of ageing, lifespan analysis can offer insight if degeneration is occurring. Furthermore, fly mutants can be examined for human disease hallmarks by histological analysis. (5) Owing to the simplicity of the fly immune system, it offers a robust model to examine the conserved function of the immune response upon infection. Typical readouts include survival, phagocytic engulfment of bacteria and colony-forming assays to measure how well flies clear the infection. (6) Complex neurological behaviours can be modelled in flies. Learning and memory assays can be performed by using a T-maze. Flies are trained to enter two chambers with two different neutral odours, and then one is then paired with an electric shock. Short- and long-term memory tests can then be conducted to test for avoidance of the chamber with the shock. Lastly, flies may be assessed for their response after mechanical stress.

et al., 2017; Rousseaux *et al*., 2016; Shulman *et al*., 2013). For example, as the most common cause of dementia, Alzheimer's disease (AD) leads to a progressive impairment in learning and memory and general cognitive decline. Pathologically, AD patient's brains are characterised by cortical atrophy and the presence of two key hallmarks: amyloid plaques made up of Aβ peptide, which results from the processing of amyloid precursor protein (APP) and neurofibrillary tangles (NFT) made up of insoluble, phosphorylated form of the microtubule-associated protein, Tau. The overexpression of either of these products causes toxicity in flies allowing for modifier screens. Ectopic expression of the most toxic species of Aβ, Aβ42, leads to amyloid pathology, learning and memory deficits and neuronal cell loss (Iijima *et al*., 2004). Aβ42 expression in the retina also causes visible degeneration, a phenotype that can be utilised in screens to uncover suppressors and enhancers that modify Aβ42-mediated toxicity (Lenz *et al*., 2013). Furthermore, genetic interaction screens in flies with Tau overexpression have elucidated modifiers that were found to be AD susceptibility genes (Shulman *et al*., 2013). In a similar vein to AD, the accumulation of misfolded proteins is observed in Parkinson's disease (PD). PD is primarily caused by loss of dopaminergic neurons causing progressive motor impairment. Unfortunately, nearly all mouse models developed based on familial PD genes fail to recapitulate this key

feature. However, the fly has proven to be a competent PD model as ectopic expression of autosomal dominant PD genes *SNCA* or *LRRK2* pathogenic mutants results in climbing deficits and loss of dopaminergic neurons (Feany and Bender, 2000; Marcogliese *et al*., 2017). Intriguingly, *SNCA* encodes α-synuclein that does not have a conserved homologue in *Drosophila*, yet expression in flies produces hallmarks of PD features including Lewy body-like aggregates, locomotor deficits and dopamine cell loss (Feany and Bender, 2000). Candidate gene approaches based on *Drosophila* models of polyglutamine (polyQ) disorders to identify targets that suppress α-syn-mediated toxicity have identified the importance of chaperones such as *HSP70*, a gene implicated in PD via genome-wide association studies (GWAS) (Broer *et al*., 2011; Lu and Vogel, 2009). In addition, flies were used to reveal that expression of a phosphor-deficient α-syn at Ser129 suppresses toxicity indicating the importance of kinase-mediated phosphorylation of α-syn (Lu and Vogel, 2009).

Huntington's disease (HD) has a robust genetic component primarily caused by cytosine–adenine–guanine (CAG) repeats in the *HTT* gene that causes polyQ expansions in the HTT protein. Other degenerative disease such as spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7 and 17, spinal and bulbar muscular atrophy (SBMA) and dentatorubral-pallidoluysian atrophy (DRPLA) result from to similar polyQ expansions in other genes. For example, CAG repeats are expanded in *ATAXIN3* that cause SCA3 (McGurk *et al*., 2015). Similar to other neurodegenerative diseases, expression of polyQ-related genes causes retinal degeneration in flies allowing a scorable phenotype for suppressor/enhancer screens. This has been done for SCA1, SCA3, HD, SBMA and other disorders (Fernandez-Funez *et al*., 2000; McGurk *et al*., 2015; Nedelsky *et al*., 2010). These studies and others in the fly have shed insight on the involvement of chaperones and the ubiquitin-proteasome system in neurodegeneration.

Flies have been a particular triumph when examining the pathways involving recessive PD genes that converge on mitochondrial quality control pathways. *PRKN, PINK1* and *PARK7* are linked to autosomal recessive, early-onset PD. *PRKN* is homologous to parkin and loss-of-function flies developed by multiple independent groups display mitochondrial abnormalities and flight muscle degeneration (Lu and Vogel, 2009). Mitochondrial dysfunction has been implicated in both human PD patients postmortem and toxin models of PD (Lin and Beal, 2006). *Pink1*-deficient flies mimic parkin-null flies. Interestingly, both mitochondrial morphology and muscle degeneration in *pink1* loss-of-function flies can be rescued by parkin expression. However, *pink1* expression fails to rescue defects in parkin-null flies, thereby putting *pink1* upstream in what is now the heavily studied and conserved Pink1–Parkin pathway (Lu and Vogel, 2009). *PARK7* encodes DJ-1, whereas the fly unusually has two paralogues, *DJ-1*α and *dj-1*β. *DJ-1*α downregulation results in oxidative stress sensitivity, which is mediated by the Akt pathway that has since been shown to be involved in a mitochondrial homeostasis pathway in parallel to *pink1/parkin* and confirmed to be involved in the Akt pathway in mammals (Martin *et al*., 2011).

Neurodevelopmental disorders

The fly has had a major role in providing insights on developmental processes. The same is true for neurodevelopment. The larval brain develops within days and undergoes an entire rearrangement defined by apoptotic and pruning pathways to eventually develop the adult CNS (central nervous system) with over 250 000 neurons. Familial forms of neurodevelopmental disorders that encompass a varying range of symptoms including intellectual developmental disability, autism spectrum disorder (ASD), seizures and cognitive impairment may be modelled in the fly. Many of the assays used in neurodegenerative disorders are also still applicable. Furthermore, flies may be assayed for 'seizure-like' paralysis by heat, electric shock or mechanical manipulation.

A common theme in neurodevelopmental disorders is a disruption of transcriptional regulators that may cause various changes to a number of genes throughout development. Rett syndrome is a leading cause of female intellectual disability and is linked primarily to perturbations in the transcriptional regulator methyl-CpG–binding protein 2 (*MECP2*) (Amir *et al*., 1999). While the fly lacks a conserved *MECP2* homologue, *MECP2* overexpression causes morphological (eye, wing vein) and motor abnormalities, making the *MECP2* fly an optimal platform for unbiased genetic screening to determine suppression pathways that may lead to targeted therapeutics (Cukier *et al*., 2008). One of the modifiers found in the study was the *UBE3A* target *pebble.* Interestingly, the vast majority of cases of Angelman syndrome (AS) are caused by loss-of-function mutations in the ubiquitin ligase *UBE3A* (Kishino *et al*., 1997). AS is characterised by developmental delay including both cognitive and motor abnormalities. Accordingly, *Drosophila ube3a* null or *ube3a* mutant flies containing equivalent AS-linked mutations display climbing deficits, abnormal circadian rhythms and long-term memory deficits (Wu *et al*., 2008). Recent genetic screening in flies has revealed that *ube3a* regulates monoamine synthesis suggesting therapeutic strategies that modulate serotonin (Ferdousy *et al*., 2011).

One of the most common forms of heritable intellectual disability and ASD is fragile X syndrome (FXS) caused by polyQ expansions in the 5′ UTR of *FMR1* leading to a silencing of the gene. Loss of function of fly *fmr1* results in neuronal architecture abnormalities that can be rescued by *FMR1* but not the other two human *FXR1P* or *FXR2P* paralogues (Coffee *et al*., 2010). This indicates conserved function of *FMR1* and enrichment for its target genes. Indeed, flies have been able to not only model a variety of neurodevelopmental disorders but also have shed insight on common themes across these disorders.

Cancer

As the genetic toolkit for the fruit fly has grown, so has interest in using the fly as a tractable model for cancer. *Drosophila* have proven to be vital for the cancer field as they have aided in identifying both oncogenes and tumour suppressors as well as uncovering complex signalling cascades. The limits to tissue overgrowth are tightly regulated by signals involving apoptosis, proliferation, the cell cycle and invasion. Loss of regulation of

these processes is hallmarks of cancer. However, it is widely thought that for cancer progression to occur, mutations must arise in both oncogenes and tumour suppressors. Activated oncogenes may stimulate apoptotic machinery, but cell death is blocked by loss of a tumour suppressor. In turn, cells continually send signals to neighbours to proliferate. Flies have offered an ability to examine the major aspects of cancers, allowing genetic control of multiple genes in a variety of tissue types. The fly provides a variety of tissues to examine cancer outcomes (Vidal and Cagan, 2006). These include the ovaries, the developing larval brain and muscle, the haematopoietic system, midgut, the developing wing and eye imaginal discs and the adult wing and eye. **See also**: **Modelling Cancer in *Drosophila*: The Next Generation**

Genetic screening in flies has identified known human cancer genes as well as novel potential disease targets (Pagliarini and Xu, 2003). One of the most well-known pathways defined in the fly is the *hippo* pathway, which is also implicated in many cancers. Activated Ras in eye clones results in overgrowth and benign 'tumours' (Pagliarini and Xu, 2003). However, loss of the fly gene *scribble* combined with activated Ras can result in invasive and malignant tumours (Uhlirova and Bohmann, 2006). Border cell migration through the egg chamber as an epithelial patch provides an *in situ* model to assay epithelium-to-mesenchyme-like transitions. Understanding the genetic control of this pathway by studying mutant phenotypes along with transplantation experiments where mutant cells invade host tissues have been successful strategies for studying conserved cancer biology (Vidal and Cagan, 2006). Indeed the most intriguing aspect of studying cancer with flies is the ability to use clonal analysis to make mutant clones surrounded by wild-type tissue to recapitulate pathogenesis. In this manner, it has been shown that increased *Myc* oncogene leads to a competitive advantage where cells out complete their neighbours by apoptosis (de la Cova *et al*., 2004).

Cardiovascular disorders

Congenital heart defects are the most common major birth defect in humans. The fly heart, called the dorsal vessel, offers a simple system for examining cardiac and muscle cell development. Over the last decade, *Drosophila* studies have revealed transcription factors involved in heart development (Bier and Bodmer, 2004). The bulk of these studies have examined specific factors that play a role in cardiac and muscle differentiation. For example, early work has shown that the conserved *mef2* gene in flies is required for muscle cell differentiation as *mef2* null flies fail to form muscle (Ranganayakulu *et al*., 1995). Years later, autosomal dominant indels in *MEF2A* were linked to coronary heart disease in humans (Wang *et al*., 2003). Continuing fly studies manipulating *mef2* in a tissue-specific manner found that *mef2* is vital for early stages of adult myoblast fusion as mutants are unable to form myotubes (Bryantsev *et al*., 2012). Another transcription factor, *hand*, is the only gene in the fly genome of the bHLH family, and is regulated by *Tinman* and *Pannier*. Loss of *hand* in flies causes robust cardiac defects and *HAND2* loss of function has recently been implicated in familial ventricular septal defect and pulmonary stenosis (Han *et al*., 2006; Sun *et al*., 2016). Other studies have shown the importance of micro ribonucleic acid (miRNA) in cardiac development. Specifically, miR1 has been shown to be a muscle-specific gene modulating both cardiogenesis and muscle gene expression and controls differentiation of cardiac and muscle cell progenitors by targeting the Notch ligand delta (Kwon *et al*., 2005). Since heart disease is also the most common contributor to mortality in adult humans, a number of groups have looked at the dorsal vessel to model cardiac ageing; the fly heart deteriorates with ageing displaying diminished stress resistance, increased arrhythmia and myofibrillar disorganisation (Nishimura *et al*., 2011). These phenotypes are mediated by potassium channel function and insulin/TOR signalling (Ocorr *et al*., 2007). In an attempt to model cardiac function directly, optical coherence tomography has been used on awake flies *in vivo*. This assay has shown that loss of fly genes, *troponin1* or *tropomysin* impair systolic heart function in flies. Furthermore, expression of the familial dilated cardiomyopathy-linked *SGCD*-S151A mutation in the fly heart causes impaired systolic function (Wolf *et al*., 2006). Since then, cardiac assays have been further refined in the fly to include heartbeat measurement, Ca^{2+} sensors and field potential recordings (these assays are discussed further in Ugur *et al*., 2016).

Inflammation and infectious disease

The immune systems of mammals and invertebrates are vastly different. Many genes involved in adaptive immunity in humans share little homology in the fly. However, much of what we know about the humoral or innate immune response including the Toll and NFκB (nuclear factor kappa B) pathways was first discovered in *Drosophila* (Lemaitre and Hoffmann, 2007). Within an open system, the circulating haemocytes of the fly act as the primary response to pathogens and wound healing. Multiple genome-wide RNAi screens have been performed in both *Drosophila* haemocyte-like S2 cells and *in vivo* to identify genes involved in antibacterial immunity via a variety of infection models. For example, in response to intestinal infection with *Serratia*, Cronin and colleagues showed that the JAK-STAT (Janus kinase/signal transducers and activators of transcription) signalling pathways determine host defence via stem cell regulation in the gut (Cronin *et al*., 2009). A similar screen has been performed in flies to determine antiviral genes that cause flies to succumb to West Nile virus (Yasunaga *et al*., 2014). *Drosophila* have also been utilised to define the mechanism underlying the pathogenicity of anthrax toxin by expression of its secreted polypeptides in the fly. This work and follow-up confirmed a conserved MAPKK (mitogen-activated protein kinase kinase) and cAMP (cyclic adenosine monophosphate) pathway for *Bacillus anthracis* infection that in downstream stages disrupts the Rab11/Sec15 exocyst (Guichard *et al.*, 2006, 2010). The results of these studies and others have revealed conserved novel antibacterial/antiviral proteins and treatment target pathways. **See also**: ***Drosophila* Innate Immunity**

Microbiome studies of the human gut have been of high interest in multiple fields including the study of human disease. Since *Drosophila* spend their lives consuming decaying food, they have evolved to respond to and benefit from microbial life within the intestine. Only recently have we begun to examine how the microbiome in flies may affect behaviour (Fischer *et al*.,

2017). Future studies may examine how microbiome diversity may influence human disease, particularly in combination with human genomic variation.

Mendelian disorders, rare and undiagnosed disease

In the genome sequencing era, a major goal has been to perform whole genome or exome sequencing on patients in attempts to provide more personalised medicine. This has been particularly informative and important in the study of Mendelian disorders. In fact, rare and undefined disorders offer a platform to discover new human disease genes and create an opportunity to use model organisms to validate novel disease variants. This requires using the humanisation strategies of replacing the fly gene with a T2A-GAL4 that causes a robust loss of function, which can be used to express the human cDNA in its place. Provided the reference human cDNA can rescue whichever phenotype loss of the fly gene has, any putative variant can then be tested for failure to rescue. This has been demonstrated recently in determining causative variants in *EBF3*-linked neurodevelopmental syndrome (#607407) and neurodegeneration (Chao *et al*., 2017; Yoon *et al*., 2017). This process of humanisation for the fly genome may inform us about the functional conservation of human genes in the fly as well as serve as readily amenable platform to test potential disease-causing variants and aid in diagnosis. Future work could employ CRISPR gene editing methods to insert a T2A-GAL4 construct within every gene.

Limitations and Summary

Modelling human disease in the fly, particularly when examining analogous tissues, can offer insight into the conserved pathways involved in form and function. However, it should be noted that there are still significant differences in fly and mammalian physiology and in some cases, signalling cascades. A common theme in the nervous system is that expression of mutant human genes can lead to robust phenotypes in the fly. This has led the field to uncovering modifiers involved in protein quality control pathways. One must still be cautious concerning overinterpretation of fly data as validation in mammalian models is vital.

The fly has proved itself as a versatile tool both genetically, and in terms of modelling a wide variety of human pathologies. Most recently, the fly may not act necessarily to model the disease but may serve as a functional platform for testing suspected disease variants in the age of personalised medicine. Owing to space requirements of this article, we apologise to our colleagues that we were unable to cover all recent techniques, models and diseases.

Glossary

CRISPR/Cas9 Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 utilizes the Cas9 endonuclease to cleave DNA guided by site specific guide RNA to edit the genome.

Humanisation Defined by genetic replacement of a fly gene with a T2A-GAL4 that is expressed in the same spatial/temporal manner as the endogenous fly gene followed by crossing to a UAS-human cDNA line of the homologous human gene.

MARCM Mosaic analysis with a repressible cell marker uses FLP-FRT recombination to label homozygous cells in a heterozygous background.

MiMIC Minos-mediated integration cassettes are a class of transposable elements that contain a gene-trap cassette, yellow+ marker and are flanked by two attP sites that may be swapped via RMCE for any other desired cassette (e.g. T2A-GAL4, protein-tag).

Transposable elements These are mobile genetic elements that can be mobilized within a host genome to disrupt genes, tag or overexpress genes.

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