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Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery

Udai Bhan Pandey and Charles D. Nichols

Departments of Genetics (U.B.P.) and Pharmacology and Experimental Therapeutics (C.D.N.), Louisiana State University Health Sciences Center, New Orleans, Louisiana

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Address correspondence to: Dr. Charles D. Nichols, Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, 1901 Perdido St., New Orleans, LA 70112. E-mail: cnich1@lsuhsc.edu.

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Abstract—The common fruit fly, *Drosophila melanogaster*, is a well studied and highly tractable genetic model organism for understanding molecular mechanisms of human diseases. Many basic biological, physiological, and neurological properties are conserved between mammals and *D. melanogaster*, and nearly 75% of human disease-causing genes are believed to have a functional homolog in the fly. In the discovery process for therapeutics, traditional approaches employ high-throughput screening for small molecules that is based primarily on in vitro cell culture, enzymatic assays, or receptor binding assays. The majority of positive hits identified through these types of in vitro screens, unfortunately, are found to be ineffective and/or toxic in subsequent validation experiments in whole-animal models. New tools and platforms are needed in the discovery arena to overcome these lim-

itations. The incorporation of *D. melanogaster* into the therapeutic discovery process holds tremendous promise for an enhanced rate of discovery of higher quality leads. *D. melanogaster* models of human diseases provide several unique features such as powerful genetics, highly conserved disease pathways, and very low comparative costs. The fly can effectively be used for low- to high-throughput drug screens as well as in target discovery. Here, we review the basic biology of the fly and discuss models of human diseases and opportunities for therapeutic discovery for central nervous system disorders, inflammatory disorders, cardiovascular disease, cancer, and diabetes. We also provide information and resources for those interested in pursuing fly models of human disease, as well as those interested in using *D. melanogaster* in the drug discovery process.

I. Introduction: Drug Discovery

A. Traditional Drug Discovery

Traditionally, the drug discovery process begins by identifying a target protein that is implicated in a certain human disease and then searching for a chemical compound that can alter the function of the disease-causing protein, generally by screening a very large library of known chemical compounds, optimizing a compound by medicinal chemistry, and then testing in animal models. This brute force approach can take more than a decade and several tens of millions of dollars to identify a single promising lead compound from chemical libraries consisting of up to several million entities. Despite the investment of significant resources, the success of finding an efficacious drug to bring to market is not guaranteed, because most drug candidates eventually fail for a variety of reasons. These include unpredicted toxicity, off-target interactions leading to undesirable side effects, and therapeutic effects not translating from traditional rodent models to humans in the clinic.

Traditional high-throughput drug screening (HTS¹) approaches are based on in vitro cell culture, biochemical assays, or receptor binding assays. To a large extent,

¹Abbreviations: AD, Alzheimer's disease; AED, antiepileptic drug; APP, amyloid precursor protein; APPL, APP-like; BS, bang-sensitive; CD, cardiovascular disease; CNS, central nervous system; DA, dopamine; FDA, United States Food and Drug Administration; FRET, fluorescence resonance energy transfer; FMR, fragile X mental retardation; FXS, fragile X syndrome; GFP, green fluorescent protein; HD, Huntington's disease; HTS, high-throughput drug screening; JAK, Janus kinase; PD, Parkinson's disease; Psn/PSN, presenilin; RNAi, RNA interference; SBMA, spinal and bulbar muscular atrophy; STAT, signal transducer and activator of transcription; UAS, upstream activation sequence; Y-27632, 4-[(1R)-1-aminoethyl]-N-4-pyridinyl-trans-cyclohexanecarboxamide.

small molecule hits identified in these assays cannot be used directly in vivo because they usually do not exhibit all the desirable characteristics for absorption, distribution, metabolism, excretion, and toxicity for the practical applications of a drug in human patients. To make these lead compounds suitable for human use, extensive medicinal chemistry optimization efforts are necessary for each lead. The failure rate of clinical products as a result of unacceptable absorption, distribution, metabolism, excretion, and toxicity characteristics is extremely high, primarily because of the poor selection of hits by test systems that have limited predictive value for clinical outcome. There are far too many examples in which HTS of several thousand to hundreds of thousands of chemicals have led to the identification of potential hits that all failed upon further testing. For example, a recent screen of 184,880 novel compounds using a "filter retardation assay" of Huntington's disease (HD) aggregates led to the identification of multiple lead compounds, including a number of benzothiazoles that inhibited polyglutamine-mediated aggregation of toxic and misfolded proteins (Heiser et al., 2002). Because riluzole, a closely related benzothiazole, had previously shown therapeutic benefit in patients with amyotrophic lateral sclerosis (Lacomblez et al., 1996), drugs from this structural class of molecules were tested for further development. In a cell culture model of aggregation, all primary hits were found to be toxic to cells, and in an animal model of HD, none of the compounds was of therapeutic value (Hockly et al., 2006).

It is often said that "all the low hanging fruit has been picked" when referring to the discovery of novel therapeutics. The number of new drugs coming to market year after year is substantially lower now than in years past. It is now clear that the paradigm has shifted from

the “one disease–one target” mentality to an understanding that nearly all diseases are multifactorial, involving many genes and proteins, each interacting with one another as well as with their environment. To take these factors into account, as well as to overcome the barrier of poor predictive value from current *in vitro* screening platforms, one would ideally perform primary drug screening directly in whole animals, where all relevant systems are present, not in isolation, but functioning together in an intact living organism that has high face validity with respect to human disease therapeutics. Traditional animal models, such as rodents, however, are a poor choice for a whole-animal primary screening platform. Primary screens examining the efficacy of many tens of thousands to hundreds of thousands of small molecules in rodents would be nearly impossible for many reasons, including the time necessary (especially for age-related diseases) and prohibitive costs. New directions outside the cell culture dish are needed in the drug discovery process to identify not only new therapeutics, but new targets as well.

B. New Directions

The fruit fly *Drosophila melanogaster* represents one such valid alternative in the drug discovery process. This review is an attempt to provide an overview of the advantages and uses of *D. melanogaster* in the drug discovery process. It is noteworthy that, as discussed in sections II and IV, key physiological processes are well conserved from the fly to humans. Further advantages of *D. melanogaster* include the extremely low cost of maintenance, propagation, and screening and the rapidity of studies in the fly compared with traditional mammal-based models. Not only can *D. melanogaster* be used in primary small molecule discovery validation, but they

can also be an important aspect of the target discovery process by taking advantage of the sophisticated genetics available in the fly (Fig. 1). Screening for novel drugs in *D. melanogaster* enables the selection of high-quality hits that already display key features such as oral or “transdermal” availability, metabolic stability, and, most importantly, low toxicity. Such features cannot yet be adequately mimicked by cell culture or biochemical assays. Here, we briefly outline the history of and basic biology of the fly, discuss therapeutic categories of human diseases in which there are currently opportunities to use the fly, and how the fly is or can be used, and we present a list of resources and information about *D. melanogaster* for those interested in pursuing the use of the fly in their own programs.

II. *D. melanogaster* As a Model Organism

A. History

The history of the use of *D. melanogaster* in modern biological sciences is a very rich one, spanning over 100 years; therefore, it is not possible to do complete justice to it here in this forum. Nevertheless, we believe that it is important to highlight a few significant aspects of this system for perspective. For more in-depth material on this subject, we recommend the following articles and references therein: Rubin and Lewis (2000), Arias (2008), Greenspan (2008), and Bellen et al. (2010). The concept that heritable traits are carried on the chromosomes was first developed in the fly, as well as many other landmark discoveries in genetics. Indeed, the Nobel Prize for Physiology and Medicine in 1994 was awarded to Ed Lewis for his pioneering research in flies defining gene structure, as well as to Eric Weischaus and Christiane Nusslein-Volhard for their studies inves-

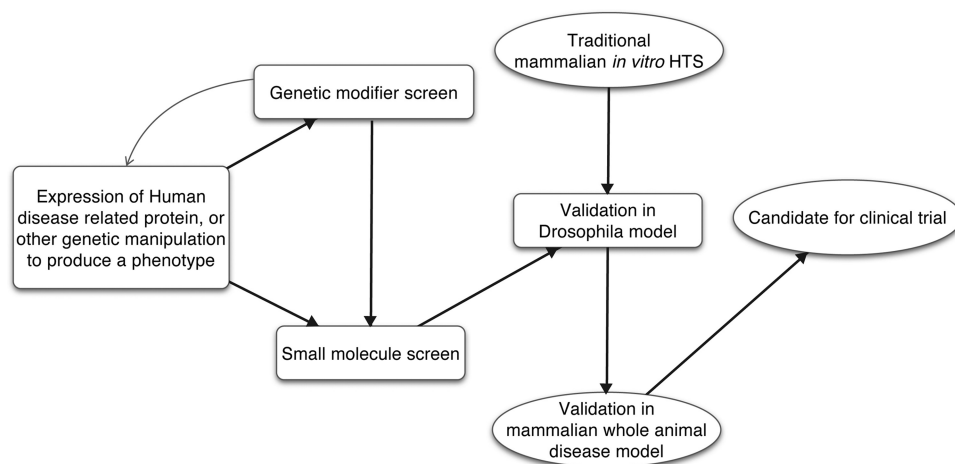


FIG. 1. *D. melanogaster* in the drug screening process. Models of human diseases are created in the fly by generation of mutants, either by mutation of the fly homolog of a human disease-related gene or by expression of the human form of the gene itself, that produce a scorable phenotype. This model can be directly screened for small molecules that rescue the phenotype or subject to genetic screens to identify modifiers of the phenotype, which represent new potential targets or models for the given disease. After initial screening, positive hits can be validated by testing in additional fly models of the disease. Significantly, these whole-animal validation studies can also be performed with the positive hits from traditional *in vitro* mammalian cell culture HTS to rapidly identify effective lead compounds. Drugs with efficacy in *D. melanogaster* models, however, will still need to be validated in mammalian whole-animal disease models.

tigating embryogenesis that identified a significant number of genes involved in all aspects of development. The vast majority of these genes have been subsequently found to be essential for normal mammalian development. In the modern era, *D. melanogaster* was the first major complex organism to have its genome sequenced (Adams et al., 2000). Not only did this event tremendously affect the fly world but also upon completion of the sequence of the human genome a few years later, the observed homologies between the two genomes underscored and strengthened its role as a model to understand human biology and disease processes. The fly continues to be at the forefront of biology, where genes, genetic techniques, and other discoveries are often elucidated first in the fly and then translated to mammalian systems.

B. Basic Biology

There are many salient features of the fly that make it such an attractive model to study. As mentioned previously, the fly genome has been completely sequenced and annotated, and encodes for a little more than 14,000 genes on four chromosomes, only three of which carry the bulk of the genome. It has been estimated that nearly 75% of disease-related genes in humans have functional orthologs in the fly (Reiter et al., 2001; Lloyd and Taylor, 2010). Overall identity at the nucleotide level or protein sequence between fly and mammal is usually approximately 40% between homologs; however, in conserved functional domains, it can be 80 to 90% or higher.

The fly has a very rapid life cycle. A single fertile mating pair can produce hundreds of genetically identical offspring within 10 to 12 days at 25°C. This is in contrast to traditional rodent models, in which only a handful of offspring are produced every 3 to 4 months. The fly may be considered multiple model organisms, each with its own specific advantages, defined by developmental stage: the embryo, the larva, the pupa, and the adult. The embryo is often used in fundamental developmental studies examining pattern formation, cell fate determination, organogenesis, and neuronal development and axon pathfinding. The larva, particularly the wandering third instar larva, is routinely used to study developmental and physiological processes as well as some simple behaviors such as foraging. The future adult structures of the fly are contained within the larva as imaginal discs, which are primarily composed of undifferentiated epithelium. Beginning in the late third instar larval phase and proceeding through the pupal phase, these structures undergo massive morphological changes that give rise to the final adult structures. The study of the molecular and genetic mechanisms underlying imaginal disc developmental processes in the pupa has provided significant insight not only to fly biology but also to human biology. Thus, the pupa is an appropriate model to investigate certain developmental processes.

The adult fly is a very sophisticated and complex organism not unlike higher organisms. The adult fly has structures that perform the equivalent functions of the mammalian heart, lung, kidney, gut, and reproductive tract. The brain of the adult fly is quite remarkable. More than 100,000 neurons form discreet circuits and neuropil that mediate complex behaviors, including circadian rhythms, sleep, learning and memory, courtship, feeding, aggression, grooming, and flight navigation. Significantly, the response of flies to many drugs that act within the CNS is similar to the effects observed in mammalian systems (McClung and Hirsh, 1998; Moore et al., 1998; Bainton et al., 2000; Nichols et al., 2002; Rothenfluh and Heberlein, 2002; Satta et al., 2003; Wolf and Heberlein, 2003; Andretic et al., 2008). The visual system of the adult continues to be extensively studied and has been crucial in understanding not only vision but also other key systems that include signal transduction pathways such as ras, and transient receptor potential channels, among many other processes (Ready et al., 1976; Nagaraj and Banerjee, 2004; Montell, 2005; Kumar, 2010).

Although there are many differences between flies and humans, the degree of conserved biology and physiology position *D. melanogaster* as an extremely valuable tool in the drug discovery process. We envision the role of *D. melanogaster* in target discovery, in primary high throughput screening, and in post-traditional HTS validation studies to effectively and rapidly identify small collections of higher quality hits from larger collections to then proceed with in more traditional mammalian models (Fig. 1). Significantly, the addition of the fly to the discovery process is predicted to enhance the rate of discovery at reduced costs to potentially identify new targets and therapeutics.

C. Genetics Relevant to Drug Discovery

Rather than discuss in depth the entire repertoire of genetic tools available in the fly, a brief overview of those most relevant to the drug discovery process will be presented here. First is the ease and cost effectiveness to generate a transgenic animal. Essentially, the cDNA/small interfering RNA or other genetic element desired is subcloned into a region between two inverted repeat elements (P-elements are the most common) on a DNA plasmid, and the entire construct along with a helper construct expressing a transposase is injected into the posterior germ cells of fly embryos. Of 100 embryos injected (taking only a couple of hours), 10 to 15% will result in a successful germline integration event, as visualized by a marker phenotype (typically red eyes) in the F2 generation. Once the initial transgenic DNA plasmid construct is made, the injection and selection process to obtain independent founder lines takes only approximately 6 weeks. There are several commercial sources available (see section VI) that will perform the whole procedure for less than \$300 per construct, once provided with the construct.

The “workhorse” of fly transgenic models is the bipartite GAL4/UAS system, first developed by Brand and Perrimon (1993). In one parental strain, promoter regions for a particular gene drive expression of the yeast transcription factor GAL4 in defined tissues. In the other strain, GAL4 response elements (UAS) are upstream of the desired transgenic element. When the two strains are mated, the progeny express the transgene in the specific tissues defined by the GAL4 promoter element. Many modifications and enhancements of this basic system have been developed to further refine tissue specificity as well as temporal expression specificity (Roman et al., 2001; McGuire et al., 2004). A very significant resource to be used in conjunction with this technology is the Vienna Drosophila Research Center collection of UAS-RNAi responder strains. They have created a collection of RNAi knockdown strains targeting ~90% of the entire fly genome and have made it available to the research community (Dietzl et al., 2007). Together, these tools make it very easy to rapidly generate models of human disease through mutation, genetic inactivation, or misexpression of fly homologs of human disease genes or human disease genes and proteins themselves.

D. D. melanogaster in Relation to Other Model Organisms: Zebrafish and Caenorhabditis elegans

Two additional model organisms are useful for drug discovery, each model providing certain advantages over the other. The selection of which model organisms to use depends on the nature of disease being studied, the scientific questions being asked, and the type of small-molecule screening procedure desired. In many instances, the smaller and genetically tractable models, such as *D. melanogaster*, *C. elegans*, or *Danio rerio* (Zebrafish), can each provide critical information about genetic and cellular process underlying certain diseases in a more rapid and cost effective manner than traditional rodent-based or in vitro studies. Here, we will only briefly highlight aspects of these two additional models for comparison with the fly.

The sequencing of the *D. melanogaster*, *C. elegans*, and Zebrafish genomes have made these small animal models more applicable and useful for the study of human diseases than they were before the “genomic revolution” (Adams et al., 2000; *C. elegans* Sequencing Consortium, 1998). Significantly, approximately 75% of human disease genes have homologs in *D. melanogaster* (Lloyd and Taylor, 2010). The worm has slightly less, at approximately 65% (Sonnhammer and Durbin, 1997). Zebrafish, being a vertebrate, is predicted to have more than the fly or the worm, with most human genes having a homolog (Langheinrich U, 2003). *C. elegans* has an extremely rapid life cycle (~4 days), is prolific, and very amenable to genetic manipulation (Teschendorf and Link, 2009). Furthermore, all 302 neurons and their connections have been precisely mapped and well stud-

ied (Teschendorf and Link, 2009). The transparent nature of *C. elegans* throughout its life cycle facilitates the use of GFP fusion proteins to visualize specific cells, neurons, and synaptic connections throughout the live animal (Link et al., 2001). It is possible to directly visualize neuronal death in living worms by the morphological appearance of vacuolated neurons (Teschendorf and Link, 2009). With respect to drug discovery, *C. elegans* has been largely employed to identify moieties related to basic cellular function in screens and experiments primarily using fluorescent-based or very simple behavioral output measurements. High-throughput screens can be performed that involve worm sorters and high density plates. For example, neurodegeneration related to Parkinson’s disease (PD) has been an active area of discovery. Live worms expressing GFP in dopaminergic neurons are easily scorable for severity of response to treatments including lesioning with 6-hydroxy-2-dipropylaminotetralin or overexpression of α -synuclein (Nass et al., 2002; Lakso et al., 2003). Both high-throughput genetic and low-throughput chemical screens have been employed to identify genetic modifiers and pharmacological treatments that block neurodegeneration, some of which have been validated for efficacy in mammalian systems (Nass et al., 2005; Marvanova and Nichols, 2007; Harrington et al., 2010).

There are, however, a number of limitations associated with the *C. elegans* as a model system compared with flies. *C. elegans* have fewer gene homologs in mammals, some families having no homologs at all (Rikke et al., 2000). It is noteworthy that many key organs and other physiologically relevant systems present in both the fly and human are absent from the worm. These include a sophisticated immune system and some organs, such as the heart. Another aspect of *C. elegans* that can be either advantageous or a limitation is that the worm does not have a male/female sexual system. Most worms are self-fertilizing hermaphrodites. Although the limited but well defined 302 neuron nervous system also has certain advantages, there is no centralized brain capable of mediating the repertoire of complex behaviors present in the fly that are relevant to human behaviors, precluding the use of the worm in screens involving anything but the most simple of behaviors.

Zebrafish as a vertebrate model system also provide many advantages for understanding molecular mechanisms of human disease such as neurodegeneration and cancer (Bandmann and Burton, 2010; Newman et al., 2011). Because zebrafish are vertebrates, most human genes have homologs, and the functional domains of many key proteins can be nearly 100% identical between homologs (although overall protein similarity levels are approximately 70% (Woods et al., 2000; Langheinrich, 2003). Zebrafish have been highly informative in studies investigating developmental processes because of their large transparent embryos that mature outside of the

mother. Additional advantages to the zebrafish system include rapid early embryonic development (although development time to the adult stage is comparable with that of mice), the presence of some organs truly homologous to humans (e.g., liver, kidney), a complete immune system (innate and adaptive), ease of drug administration, and a lower infrastructure cost than rodents. The small size of zebrafish allows them to be housed in a small lab space, and it is possible to get a large number of progeny (200–300 new progeny per week per pair) within a short period of time (Meeker and Trede, 2008). Unlike *C. elegans*, zebrafish display complex behaviors relevant to humans. It is noteworthy that zebrafish have been successfully used in drug discovery and chemical screening processes (Tsang, 2010). Early studies used medium-throughput approaches examining the mutagenic effects of chemicals on embryo development (Peterson et al., 2000). Examples of more recent studies include high-throughput primary screens to identify small molecules that inhibit fibroblast growth factor receptor signaling (Molina et al., 2007) and secondary validation screens of traditional HTS hits to identify modifiers of circadian activity (Hirota et al., 2010).

Despite certain attractive features of the zebrafish model, there are also limitations. Although the development of genetic tools available for use in the zebrafish is progressing, they are arguably not nearly as advanced as those currently available for the fly or the worm. Furthermore, although more inexpensive than rodent facilities, zebrafish do require substantially more infrastructure- and maintenance-associated costs compared with both flies and worms.

III. Considerations

A. Differences between Fly and Human

Although the bulk of this review is dedicated to describing the conserved biology between fly and human and how these similarities can be exploited in the drug discovery process, the fly is *not* a miniature person. Fundamental processes can be shared, but their implementation can be and is often very different. With regard to basic physiological and cellular processes, such as glucose utilization or receptor signaling pathways, or where the underlying cause of a human disease may be due to dysfunction of only a single gene or protein, fly models can have high degrees of conservation and face validity, facilitating primary screening and interpretation of results. For more complex processes and modeling multifactorial human diseases, the corresponding fly models usually are only able to model certain aspects of the disease, and interpretation of results are more complicated. Whereas this is especially true for models of behaviors, other contributing factors can include significant differences in physiology that produce simpler or different phenotypes in the fly. Although *D. melanogaster* models can be informative in the discovery pro-

cess, having a well defined hypothesis and a thorough understanding of the limitations of the fly are absolutely critical for success.

With respect to drug discovery, a key consideration to take into account are potential differences in the pharmacokinetics and pharmacodynamics of small molecules, which may produce significant discrepancies in drug levels and tissue distribution profiles between mammal and fly. For CNS discovery, there may be blood-brain permeability differences (Stork et al., 2008; Mayer et al., 2009). Another very important issue is toxicity. Because of metabolic differences, some drugs may be toxic in flies that are not in humans and vice versa, although there seems to be a strong correlation of toxicity between the two species (Rand, 2010). Because of all these factors, and more, it is emphasized here that *D. melanogaster* can be used only as a screening platform for target discovery, primary small-molecule screening, or postscreening validation to narrow down a large pool of potential drug candidates to a much smaller pool of lead compounds that it will be absolutely necessary to validate using traditional mammalian models. Nevertheless, the incorporation of *D. melanogaster* in target discovery and HTS is predicted to enhance the rate of discovery by reducing the time necessary to identify a small collection of potentially more effective lead compounds for final validation than by traditional methods by virtue of performing the discovery phase in a whole animal following a systems-based approach.

B. Drug Delivery Issues

A natural question to ask is: “How do you give drugs to a fly?” For embryos, drugs can be administered via permeabilization (Rand et al., 2010). For larva, drugs are usually added to the solid media in which they grow for long exposures or in a dilute solution of yeast paste for shorter exposures. For adult flies, there are numerous routes of drug administration (Fig. 2). Drugs can be presented as a vapor (e.g., ethanol and cocaine) (McClung and Hirsh, 1998; Moore et al., 1998); in the food itself or from a sucrose/drug-saturated filter paper (Nichols et al., 2002); drug can be injected or dropped directly onto the exposed nerve cord of decapitated flies (Torres and Horowitz, 1998); drug can also be injected into the abdomen, where it quickly diffuses throughout the organism (Dzitoyeva et al., 2003). Potential issues determining route of administration include the taste of a drug: if a drug tastes bad, a fly is likely to not eat it. To determine whether the presence of a drug influences food intake, there are simple feeding assays that can be performed (Ja et al., 2007). If it is necessary for an unpalatable drug to be ingested, it can be included with a rewarding substrate (e.g., sucrose, banana, or yeast paste). The most high-throughput method is to dissolve drug either in normal food substrate, or agarose + sucrose (to ensure that no ingredient in the food will interfere with drug action or absorption), and aliquot into

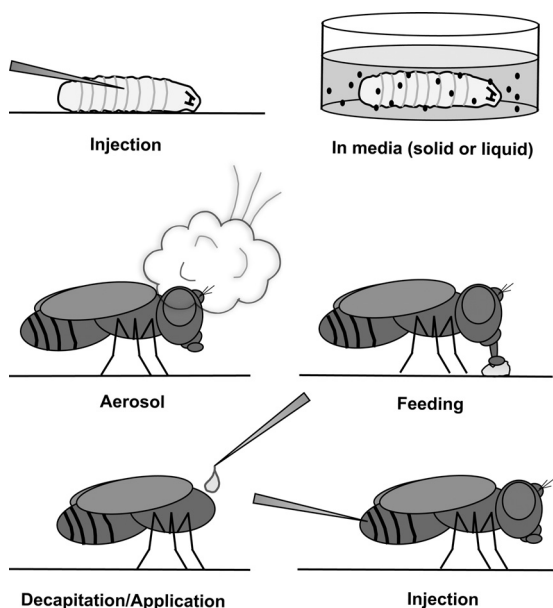


FIG. 2. Routes of drug administration. For larva (top), drug can be directly injected or drug can be mixed with media. Media can be either solid or liquid with 2% yeast paste to encourage feeding behavior. Adults can have drug delivered as an aerosol or gas, as a mixture with food substrate, as a direct application to exposed nerve cord, or as an injection. Drug administration through feeding generally has the highest throughput.

wells of a high-density plate that will contain individual animals. Physiologically effective concentrations can vary from 0.01 to 100 mM in the feeding substrate, although most studies examining the effects of drugs are in the range of ~1 to 10 mM. It must be emphasized that these are concentrations in the food; actual physiological concentrations will be much lower, and it may be necessary to examine *in vivo* concentrations using high-performance liquid chromatography or mass spectrometry (Kuklinski et al., 2010). It is recommended that pilot studies be performed examining three different concentrations of a known effective drug at log dilutions in the feeding substrate (0.03, 0.3, and 3.0 mM) for efficacy in a particular assay and to choose an appropriate concentration based on those results for the full screen.

There are different strategies to be considered when feeding drug to adults. One method is to starve the flies for ~16 to 18 h, and then place the flies on food substrate + drug for a few minutes. The flies will generally consume a large bolus dose of the drug. Advantages of this method include observation or measurement of the acute effects of a drug. Disadvantages include significant variability in dosage among flies as a result of various body sizes and amounts of drug ingested, as well as relatively low throughput. In our experience, drugs administered by this method begin to show behavioral effects within 10 min; maximum effects last from 15 to 60 min after feeding and full recovery occurs at 2 to 5 h. The second method is to maintain the fly on food substrate + drug for longer than 24 h, which allows for steady-state levels to be achieved before testing.

This method is the highest throughput and can allow for large populations to be administered drugs. Disadvantages include possible adaptive mechanisms to prolonged exposure, such as down-regulation or desensitization of target genes or proteins. If flies are removed from the food + drug before any testing, metabolism and rate of elimination of the drug may need to be accounted for, depending on the assay, when interpreting the data.

C. Throughput

High-throughput screening by traditional methods usually involves massive parallel analysis of the effects of small molecules from a large library of 100,000 compounds or more on mammalian cells in culture in 384-well plates. An example of this is the fluorometric imaging plate reader assay, often used for the identification of molecules that alter the function of certain G-protein-coupled receptors (Sullivan et al., 1999). Throughput in *D. melanogaster* terms, for the most part, does not nearly approach that of HTS in mammalian cell culture systems. Whereas high throughput in some fly screens may approach 10,000 small molecules per month, most screens are on the order of 500 to 1000 small molecules per month. Although fly models have much lower throughput in general, one must keep in mind that, ultimately, the raw number of compounds screened from a library is not important in the discovery process; the number of quality hits resulting from the screen is the measure of success. Traditional brute-force HTS approaches can identify many “positive hits” from a large library in a short time, but the overall quality of these hits from a therapeutic standpoint is often quite poor, and significant resources must be expended to further develop the molecules before further testing in whole animals, at which point most lead candidates still will fail as a therapeutic (Gosai et al., 2010). The potential advantage of using the fly in the initial discovery process, regardless of raw throughput, is the identification of higher quality hits from fewer compounds screened. Because screening will be performed directly in the living animal, examination of the effects of the drug at the organismal level are built into the primary screen, thus significantly reducing postscreening costs to identify quality leads from the initial candidate pool. For example, drugs that seem safe in mammalian cell culture often produce unpredicted toxicity once tested in expensive rodent experiments. Many of these types of hits would not be selected for in fly screens because they would kill the fly. Regardless, if the higher throughput of traditional HTS is desired, then the fly can still perform a valuable role as a cost-effective and highly informative secondary screen on the positive hits to streamline the pool of candidates to those of higher quality before moving the entire collection to expensive rodent studies.

The actual throughput of *D. melanogaster* screens varies depending on the assay and to the degree to which it can be automated (Table 1). Although there are a number of different assays, only a few of the more prevalent ones currently used are described here to give an overall idea of what is possible. The higher throughput assays primarily depend on fully automated scoring of a visible phenotype, either live/dead, or a visible marker. Potentially one of the highest throughput quantitative strategies involves measuring the fluorescence of markers in embryos by methods similar to flow cytometry (Pulak, 2006). The same sorting technology can also be used to seed embryos into 348- or 96-well plates containing substrate and drug. Scorable HTS phenotypes suitable for automation include viability, both at the larval (do larva develop?) and pupal stages (do viable adults emerge from pupa?), as well as measurement of fluorescent markers (i.e., GFP-tagged proteins). Semiautomated and manual scoring of emerged adults are somewhat lower in throughput but are still able to screen thousands of drugs per week. These could involve, for example, examination for overt normal development or roughness of the eye. Lower throughput assays likely to be more relevant for validation of leads would include those in which a more detailed analysis of the fly is required (e.g., microscopic analysis of certain organs of the fly or biochemical analysis of the fly for levels of enzyme activity).

Behavioral assays, depending on the degree of automation, can also vary throughout from medium to low. The highest involve simple measurement of locomotor and circadian activity of individual flies in small glass capillary tubes by photo beam breaks (DAMS; Trikinetics, Waltham MA). Here, adult flies are loaded into tubes containing a food substrate and drug at one end that are placed into a 32-tube array. A single computer can measure locomotor activity in

hundreds of tubes simultaneously. A small facility could screen 5000 to 10,000 small molecules in 2 to 3 months by this method. Lower throughput semiautomated methods include learning and memory assays and social interaction assays. For learning and memory, conditioned stimulus training can be automated to simultaneously train 8 to 32 small populations of flies for subsequent testing (Scott et al., 2002). A single technician and a 16- to 32-channel trainer could achieve a throughput of 25 to 50 drugs per week. Social interaction assays include aggression and courtship. Although subtle aspects of each of these interactions require a human observer, some of the more important interactions can be automated by video tracking software. For example, analysis software can simultaneously examine locomotor activity and interactions of up to 96 video channels in real time that include aspects of both aggression and courtship (Noldus, Wageningen, NL) (Branson et al., 2009; Dankert et al., 2009).

IV. Therapeutic Areas and Opportunities to Use *D. melanogaster* in Drug Discovery

A. Central Nervous System

1. *Neurodegeneration.* Neurodegenerative diseases are caused by progressive loss of specific neurons and are mostly age-related human diseases with significant pathological and clinical similarity. Persons who will develop neurodegenerative diseases are generally asymptomatic during the development of the nervous system. Many late-onset neurodegenerative diseases, including PD and HD, are associated with the formation of intracellular aggregates of toxic proteins (Taylor et al., 2002). The identification of mutations associated with familial cases of many of these neurodegenerative diseases has highlighted the significance of these pathological features and allowed investigators to develop in vitro and in vivo model systems to determine the cellular and molecular abnormalities associated with mutant gene product in many neurodegenerative diseases. These models proved to be very helpful in determining the biochemical and genetic alterations in neuronal tissues and understanding how mutant proteins cause damage to specific sets of neurons leading to distinct clinical phenotypes. A consensus has emerged regarding an underlying mechanism that contributes broadly to this class of diseases. Specifically, some proteins are more prone to misfolding into disease-causing pathological conformations that assemble into aggregates and acquire neurotoxic properties. It is believed that neurodegenerative diseases ensue when the production of neurotoxic proteins exceeds the cell's capacity for disposing of them or when neurotoxic proteins evade quality-control surveillance altogether. This concept predicts that it may be possible to develop novel approaches for treatment based on a

TABLE 1
Throughput in *D. melanogaster* models

Stage	High Throughput	Medium Throughput	Low Throughput
Larvae	Lethality Body Size Necrotic Patches	Olfactory	Locomotor defect Body wall contraction Body wall muscle
Adult	Lethality Flight ability Body size Stress test Anesthesia response	Body weight Sleep, arousal, and rest behavior Fecundity Aggression Wing expansion behavior	Response to pain Life span Retinal degeneration Climbing assay Phototaxis Rotorod test Electrophysiology Prepulse inhibition Courtship behavior Feeding behavior Learning and memory behavior Response to pain Seizure behavior Visual discrimination

greater understanding of the cellular mechanism responsible for disposing of unwanted proteins.

Despite the significant contribution of human genetic studies in the identification of new genes associated with familial forms of neurodegenerative diseases, studies on human patients are of limited use for elucidating the signaling pathways and cellular processes underlying the neurodegenerative process. Often the rapid speed of the discovery of disease-causing genes is not matched by the speed of our understanding of the manner by which these mutations lead to the clinical symptoms of the disease and the mechanism of the disease progression. In addition, both ethical and technical problems pose a limit on types of genetic analysis that can be performed in human patients to determine genetic relationships among disease genes and to delineate signaling pathways. Most human neuropathological investigations use postmortem tissues, such as brain and spinal cord tissues, that almost never reflect the earliest pathologic events at the presymptomatic stage. Hence, animal models, especially *D. melanogaster*, present excellent alternatives for studying neurodegenerative disease mechanisms from early initiation events to the terminal stages.

Nevertheless, there are limitations to be aware of with fly models of neurodegeneration. *D. melanogaster* models often show striking phenotypes at early developmental stages, such as the larval, pupal, or early adult, in contrast to their human counterpart diseases that are mostly of late onset and start in the sixth or seventh decade of life (i.e., age 50–69 years). Furthermore, many of the *D. melanogaster* models rely on overexpression of the human disease-causing genes in *D. melanogaster* eyes, using eye degeneration (rough eye) as a measure of effect. Although *D. melanogaster* eyes and photoreceptor neurons have proven to be a good tool for examining the overt toxic effects of individual human disease-causing genes, eyes do not mimic the human brain with its complex circuitry and pathophysiology. *D. melanogaster* also have a much simpler immune system than mammals, limiting the study of the role of neuroinflammation in degenerative diseases. It is noteworthy that there are several significant anatomical differences between fly and human brain. For example, the fly brain has no substantia nigra, which is relevant to understanding how clinical features mediated by dopaminergic neuron loss in Parkinson's disease correlate with behavioral phenotypes. Cellular and molecular processes can also be very different in *D. melanogaster* and humans, and one or several key molecule(s) involved in mediating a disease-specific pathway could be missing in flies (e.g., α -synuclein), and there is a risk that the lessons we learn from the *D. melanogaster* model might not be biologically relevant to human disease pathways.

In the following subsections, several models of human neurodegenerative disease and their potential in the discovery arena are presented (Table 2). Each of these

models shares several phenotypic commonalities in the fly, such as retinal degeneration, locomotor defect, wing phenotype, climbing defect, and reduced lifespan. Therefore, drug discovery assays aimed at identification of therapeutics for the neurodegeneration diseases discussed below can all essentially use protocols examining these common phenotypes. For example, rescue of rough eye phenotype, rescue of locomotor and climbing deficits, and restoration of normal activity. Because of their shared screening assays, our discussion of neurodegenerative models focuses on the pathophysiology of the model and not the screening process.

2. Alzheimer's Disease. Alzheimer's disease (AD) is the most common neurodegenerative disease and is characterized by progressive impairments in memory and cognitive abilities with a typical late age of onset, although the onset can be as early as fourth decade of life (i.e., age 30–39 years) in the familial forms. The disease is characterized pathologically by selective atrophy of the hippocampus and the frontal cerebral cortex. Amyloid plaques and neurofibrillary tangles are the hallmarks of AD. The main components of amyloid plaques are the $A\beta$ -40 and $A\beta$ -42 peptides, which are generated by proteolysis of the amyloid precursor protein (APP) via the action of β - and γ -secretase enzymes. β -Secretase activity is provided by the β -site APP-cleaving enzyme, whereas γ -secretase activity depends on a protein complex consisting of presenilin (Psn), nicastrin, aph-1, and pen-2. It is noteworthy that autosomal dominant mutations in APP, PSN-1, and PSN-2 can accelerate the age of disease onset and progression in familial AD cases. These mutations promote the generation of amyloidogenic $A\beta$ peptides, and impairment in the trafficking of APP into protein degradation pathways may underlie the pathological accumulation of $A\beta$ in several late-onset familial AD cases. These findings further support the amyloid hypothesis, which postulates that the accumulation of $A\beta$ peptide is the initial event in the disease pathogenesis that may underlie synaptic failure, thereby resulting in the remarkably pure impairment of cognitive function.

Most of the genes implicated in AD pathogenesis have *D. melanogaster* homologs; e.g., the fly homolog of human APP is known as APP-like or APPL. Flies deficient for APPL demonstrate a behavioral abnormality that can be strongly suppressed by expression of a human APP transgene, indicating functional conservation between *D. melanogaster* APPL and human APP (Luo et al., 1992). As expected, however, there are some dissimilarities; *D. melanogaster* APPL lacks the amyloidogenic $A\beta$ peptide sequence at the C terminus, and it remains unclear whether APPL is processed in vivo like human APP. In *D. melanogaster*, the γ -secretase complex components are conserved and have been clearly implicated in the processing of Notch signaling pathways (Struhl and Greenwald, 1999; Ye et al., 1999). The γ -secretase- and presenilin-inhibiting compounds have been shown

TABLE 2
Genetic models of neurodegeneration

Diseases/Gene	Invertebrate or Animal	Phenotypes	References
Alzheimer's Disease β -Amyloid protein	<i>C. elegans</i>	Progressive paralysis, cytoplasmic protein accumulation, fibrillar amyloid formation	Link, 1995; Fay et al., 1998; Drake et al., 2003; Wu et al., 2006; Hornsten et al., 2007; Hassan et al., 2009
	<i>D. melanogaster</i>	Eye degeneration, Accumulation of amyloid plaques, reduced life span, locomotor defect, and vacuolation of the brain	Finelli et al., 2004; Crowther et al., 2005; Luheshi et al., 2007
	Zebrafish	Reduced body length, short and curly tail, defective convergent-extension movements in embryos	Joshi et al., 2009
Presenilin	<i>C. elegans</i>	Defects in neurite morphology, temperature memory, egg laying	Wittenburg et al., 2000
	<i>D. melanogaster</i>	Pupal lethality, dorsoscutellar bristle duplications, wing notching and wing vein defects	Seidner et al., 2006
	Zebrafish	Decreased cell proliferation and de novo neurogenesis, Irregular delineation of somites	Nornes et al., 2003; Van Tijn et al., 2009
Tau	<i>C. elegans</i>	Age-dependent progressive neurodegeneration, accumulation of insoluble tau; reduced lifespan, age-dependent progressive impairment in touch response, embryonic lethality and mechanosensory defect	Kraemer et al., 2003; Miyasaka et al., 2005; Gordon et al., 2008; Feuillette et al., 2010
	<i>D. melanogaster</i>	Eye degeneration, disruption of the microtubular network at presynaptic nerve terminals, axonal degeneration, neuromuscular junctions morphological defects	Williams et al., 2000; Whittman et al., 2001; Jackson et al., 2002; Mudher et al., 2004; Nishimura et al., 2004; Chee et al., 2005; Blard et al., 2007; Chen et al., 2007
	Zebrafish	Pathological hyperphosphorylation, conformational changes, and tau aggregation	Paquet et al., 2009
Parkinson's Disease α -Synuclein	<i>C. elegans</i>	Mitochondrial stress, dopaminergic degeneration, developmental defect, upregulation of dopamine synthesis, redistribution of dopaminergic synaptic vesicles	Lakso et al., 2003; Springer et al., 2005; Ved et al., 2005; Kuwahara et al., 2006; Karpinar et al., 2009; Hamamichi et al., 2008; Kuwahara et al., 2008; van Ham et al., 2008; Settivari et al., 2009; Cao et al., 2010
	<i>D. melanogaster</i>	Age+dependent loss of dopaminergic neuron and progressive climbing defect	Feany and Bender, 2000; Auluck and Bonini, 2002; Auluck et al., 2002; Coulom and Birman, 2004; Pesah et al., 2005
	Zebrafish	Zebrafish homologs of human α -synuclein are known but no animal model published yet	Sun and Gitler, 2008; Chen et al., 2009
Parkin and Pink	<i>C. elegans</i>	Hypersensitivity toward proteotoxic stress conditions, Parkin insolubility and aggregation	Springer et al., 2005
	<i>D. melanogaster</i>	Dopaminergic neuron loss, age-dependent motor deficits, reduced lifespan, locomotor defects, male sterility and mitochondrial pathology	Greene et al., 2003; Haywood and Staveley, 2004; Pesah et al., 2004; Cha et al., 2005; Sang et al., 2007
	Zebrafish	Dopaminergic neuron loss, reduced mitochondrial respiratory chain complex I activity, severe developmental defect	Anichtchik et al., 2008; Flinn et al., 2009; Xi et al., 2010
Triplet repeat expansion diseases Huntington's disease	<i>C. elegans</i>	Huntingtin-positive cytoplasmic aggregates, sensory process degeneration, axonal swelling, mechanosensory defects and perinuclear huntingtin aggregates	Faber et al., 1999; Parker et al., 2001, 2005; Brignull et al., 2006
	<i>D. melanogaster</i>	Axonal transport defect, lethality, neurodegeneration, behavioral and electrophysiological defects	Gunawardena et al., 2003; Romero et al., 2008
Spinal and bulbar muscular atrophy	Zebrafish	Massive neuronal apoptosis, small eyes and heads and enlargement of brain ventricle, lower jaw abnormalities; defect in iron utilization and development	Lumsden et al., 2007; Henshall et al., 2009
	<i>C. elegans</i>	None	Takeyama et al., 2002; Pandey et al., 2007a,b; Nedelsky et al., 2010
	<i>D. melanogaster</i>	Accumulation of expanded polyglutamine containing androgen receptor, protein aggregation, eye degeneration, locomotor defect	
Fragile X syndrome	Zebrafish	None	
	<i>C. elegans</i>	None	
	<i>D. melanogaster</i>	Eye degeneration, age-related cognitive impairment, abnormal circadian rhythms, courtship behavior defect, lethality, defect in synaptogenesis, spermatogenesis	Wan et al., 2000; Zhang et al., 2001; Dockendorff et al., 2002; Morales et al., 2002; Jin et al., 2007; Sekine et al., 2008; Sofola et al., 2008; Choi et al., 2010
Zebrafish	Abnormal axonal branching, cardiomyopathy, muscular dystrophy	Tucker et al., 2006; Van't Padje et al., 2009	

to induce developmental defects in *D. melanogaster* remarkably similar to those caused by genetic reduction of the notch signaling pathway (Micchelli et al., 2003). To date, there are no published studies aimed at identifying novel potential drugs for treating AD in the *D. melanogaster* model system through screening processes. Development of invertebrate models, especially *D. melanogaster* models of AD, provide excellent tools for performing drug screens to identify small molecules that can suppress the toxicity associated with A β accumulation and modulate the γ -secretase activity.

3. Parkinson's Disease. Parkinson's disease is the second most common age-related neurodegenerative disease and is clinically characterized by muscle rigidity, resting tremor, bradykinesia, and postural instability. PD is caused by degeneration of dopaminergic neurons in the substantia nigra region of the brain. A pathological hallmark of the disease is the formation of Lewy bodies, intracytoplasmic inclusions that are composed of α -synuclein and ubiquitin, among other proteins. Most cases of PD are sporadic with no known cause. Several familial PD cases have been identified and are caused by mutations in genes, including α -synuclein (Polymeropoulos et al., 1997), Parkin (Kitada et al., 1998), DJ-1 (Bonifati et al., 2003) PINK1, (Valente et al., 2004), LRRK2 (Paisán-Ruíz et al., 2004), and ubiquitin C-terminal hydrolase-1 (Ragland et al., 2009). Of these six PD-associated genes, α -synuclein and Parkin are the most well studied genes. Two missense mutations, A53T (Polymeropoulos et al., 1997) and A30P (Krüger et al., 1998), as well as genomic duplication and triplication of the α -synuclein gene (Singleton et al., 2003; Ibáñez et al., 2004) have been identified as causes of autosomal-dominant forms of familial PD. Parkin mutations were identified in families with autosomal recessive juvenile parkinsonism (Kitada et al., 1998), and further investigations have indicated that parkin functions as an E3 ligase (Giasson and Lee, 2003; Hattori and Mizuno, 2004; Moore et al., 2005), although additional roles in microtubule-based transport (Ren et al., 2003) and regulation of DA transporter activity have been suggested (Jiang et al., 2004). It has been hypothesized that loss of E3 ligase activity is involved in the pathogenesis of parkin-linked PD. Mutations in the LRRK2 gene have been identified as other common genetic causes of PD (Paisán-Ruíz et al., 2004; Zimprich et al., 2004). The most common mutations in LRRK2 are G2019S (Lesage et al., 2006) and G2385R (Tan and Schapira, 2008). LRRK2 is composed of multiple domains, including a GTPase domain and a kinase domain capable of exhibiting GTP-dependent phosphorylation activity (West et al., 2005). It has been shown that the disease-associated mutations of LRRK2 can increase its kinase activity and thereby its toxicity (Smith et al., 2006; West et al., 2007). DJ-1 encodes a highly conserved protein belonging to the ThiJ/PfpI superfamily. There are rare mutations reported in the DJ gene, and it has been suggested that

DJ-1 is a rare cause of PD (Bonifati et al., 2003). A list of reported fly and other small animal models of PD is provided in Table 2.

4. Triplet Repeat Expansion Diseases. At present, 22 different neurological diseases are known to be caused by expansion of triplet repeats in the human genome. In 1991, two repeat expansion mutations, fragile X mental retardation syndrome (FMR1) and spinal and bulbar muscular atrophy (SBMA), were reported to produce disease phenotypes by expanded poly-amino acid tracts. The clinical phenotypes of triplet repeat expansion diseases depend on the context of the protein where the repeat expansions occur (La Spada and Taylor, 2003). Polyglutamine diseases are caused by mutations that lead to hyperexpansions of unstable CAG repeats, which are translated as glutamine in normal functioning proteins. Polyglutamine diseases are due to single-gene defects and were the first neurodegenerative models successfully created in *D. melanogaster* to use human transgenes and were generated by Nancy Bonini at the University of Pennsylvania. Polyglutamine diseases demonstrate several characteristic features in patients, such as nuclear inclusions containing the mutant protein, repeat length inversely correlated with age of onset, and age-dependent motor neuron degeneration and impairment. There are several *D. melanogaster* models of triplet repeat expansion diseases, including fragile X mental retardation with overexpression of the *FMR1* gene with various CAG repeat lengths, HD using expression of truncated wild-type and mutant forms of huntingtin/htt (Jackson et al., 2002), SCA 3 or Machado-Joseph disease expressing truncated ataxin 3 using different glutamine repeat lengths, and SBMA by expressing the human androgen receptor gene with different polyglutamine repeat lengths (Pandey et al., 2007a; Batlevi et al., 2010). All of these models demonstrated that increased poly-Gln expansion led to increased severity of degeneration, age-dependent degeneration, and repeat length-dependent protein aggregation (La Spada and Taylor, 2010). These models provided a platform to demonstrate that human disease genes can yield parallel neurodegenerative effects in *D. melanogaster*. It is noteworthy that a few studies also found that poly-Gln expression in glia can cause lethality and neurodegeneration.

5. Sleep. According to a recent Institute of Medicine report (<http://www.iom.edu/sleep>), at least 40 million Americans suffer from chronic, long-term sleep problems and an additional 20 million people experience occasional sleep disturbances. Sleep disorders account for an estimated \$16 billion in medical costs each year, although the indirect costs due to lost productivity and other factors are largely unknown and probably much greater compared with the medical costs. Therefore, this is a very attractive area for drug discovery. *D. melanogaster* exhibits many of the behavioral characteristics of mammalian sleep, enabling the use of powerful genetic approaches to understand conserved fundamental as-

pects of sleep. As do humans, flies have a circadian activity cycle. They have a morning bout (lights on) of activity, followed by a mid-afternoon siesta period of inactivity, a late afternoon peak of activity, and relative inactivity during the night (lights off) (Cirelli, 2009). The behavior and neurological function of flies in longer bouts of inactivity more closely resemble sleep in mammals than quiet rest (Shaw et al., 2000). Significantly, wake-promoting agents such as modafinil and caffeine have similar effects in the fly, as do sleep-promoting agents such as antihistamines (Shaw et al., 2000; Hendricks et al., 2003; Andretic et al., 2008).

Over the past several years, *D. melanogaster* studies have led to the identification of novel genes (for example, *Shaker* and *sleepless*) and molecular pathways that can modulate sleep and candidate brain regions known to function in circadian regulation as well as learning and memory. *Shaker*, identified with a mutational ethylmethane sulfonate screen, encodes for the α -subunit of a tetrameric potassium channel that mediates a voltage-activated, fast-inactivating I_A current. *Shaker* loss-of-function mutant flies sleep only 2 to 4 h every day rather than 8 to 10 h (Schwarz et al., 1988). Learning and memory in these shaker mutant flies is significantly impaired, and lifespan is reduced (Schwarz et al., 1988; Cirelli et al., 2005). In contrast, *sleepless* was identified by a different approach called insertional mutagenesis (Koh et al., 2008). The *sleepless* flies, similar to the *Shaker*-null mutants, sleep only 2 h/day (significantly less than age-matched control flies), mainly because of a decrease in sleep episode duration. The *sleepless* gene encodes for a glycosylphosphatidylinositol-anchored protein with unknown function (Koh et al., 2008).

It has been demonstrated that *D. melanogaster* sleep patterns change with changes in physiology and aging. It has been shown that sleep becomes more fragmented in older flies like sleep patterns change in humans with age (Koh et al., 2006). Young flies have long, uninterrupted sleep bouts that occur mostly at night. However, sleep in older flies becomes more evenly perturbed across the 24-h day. Correlated with changes in sleep patterns during aging are changes in the strength of circadian rhythms, which suggests that the circadian clock exerts some influence over sleep consolidation. The rate of decline in the strength of circadian activity with the aging process can be altered by temperature and exposure to oxidative stress-causing agents such as paraquat (Koh et al., 2006).

Although drug discovery screens for sleep-related therapeutics have not been reported in the literature, all the tools are in place for such an initiative. Rihel et al. (2010) have successfully developed and applied a high-throughput, quantitative screen for small molecules that could alter the locomotor behavior of larval zebrafish. This is an important step toward not only identifying and characterizing psychotropic drugs involved in locomotor behavior in a whole organism but also to-

ward dissecting the pharmacology of complex behaviors. One direction for discovery in flies could be to identify wake-promoting agents either in normal animals or in genetic models exhibiting excessive sleep. Another direction could be to identify novel sleep-promoting agents by using normal flies or genetically altered flies that have reduced sleep and assaying for increased sleep. In each strategy, HTS could be performed using arrays of photo beam-based activity arrays and software to measure circadian activity, as discussed in detail in section IV.A.5.

6. Seizure Disorders. Epilepsy was one of the first brain disorders to be described (Goldenberg, 2010), affecting more than 2 million people in the United States. Similar to all animals with complex nervous systems, including humans, electrical shock delivered to the *D. melanogaster* brain elicits seizure-like activity (Pavlidis and Tanouye, 1995; Lee and Wu, 2002). Therefore, *D. melanogaster* has been developed as a model to study seizure disorders.

There is a collection of 11 seizure-sensitive *D. melanogaster* mutants, also known as bang-sensitive (BS) paralytic mutants, that recapitulate key features of human seizures. Seizure-like behaviors prominent in these mutants becomes more obvious after a mechanical shock, such as a tap of the culture vial on the bench top (a “bang”). BS mutants display seizure-like behaviors characterized by initial seizure, temporary paralysis, and recovery seizure (Benzer, 1971; Ganetzky and Wu, 1982). The BS behavioral phenotype is fully penetrant, with electrophysiological seizure thresholds usually below 7 V (Kuebler and Tanouye, 2000). Normal flies, however, never show a BS behavioral phenotype and have electrophysiological seizure limits greater than approximately 35 V (Kuebler and Tanouye, 2000). It is noteworthy that there are several noncanonical BS mutants recently identified, including *couch potato* (*cpo*) and *kazachoc* (*kcc*). Unlike most of the original BS mutants, the BS phenotype in these noncanonical BS mutants are incompletely penetrant, and their seizure thresholds tend to be somewhat higher (11–16 V) but still significantly lower than wild-type levels (Kuebler and Tanouye, 2000).

Although the BS mutant seizure physiology resembles that observed in mammals, the BS genes do not, however, correspond to known mammalian genes involved in seizure disorders. Nevertheless, there are significant similarities between human seizures and *D. melanogaster* seizure models, providing support for the utility of the *D. melanogaster* model system for drug discovery. The importance of the *D. melanogaster* seizure model has been further strengthened by the fact that seizure-like activity spreads through the fly CNS along particular pathways that are dependent on functional synaptic connections and recent electrical activity, as do seizures in humans. Seizure-like activity in flies can also be spatially segregated into particular regions of the

CNS. It is noteworthy that *D. melanogaster* seizure phenotypes can be ameliorated by human antiepileptic drugs (AEDs) such as sodium valproate and phenytoin (which act as sodium channel blockers), gabapentin (a calcium channel blocker), and potassium bromide (a chloride channel blocker) (Kuebler and Tanouye, 2000; Tan et al., 2004; Song and Tanouye, 2006). Other therapeutics, however, including carbamazepine, ethosuximide, and vigabatrin do not have efficacy in the BS mutant strains (Reynolds et al., 2003). It is noteworthy that the anticonvulsant lamotrigine has been found to extend lifespan of *D. melanogaster* (Avanesian et al., 2010). Consistent with the mechanism of action of AEDs that primarily target sodium channels, it has been shown that mutations in sodium channels that decrease conductance can suppress seizures in flies (Reynolds et al., 2003; Tan et al., 2004).

The drug-screening strategy for seizure disorders can potentially be divided into two steps. In the first step, a library of drugs can be delivered through either the feeding or the larval bathing methods to identify effective compounds that rescue seizure-like phenotypes or paralysis. Subsequently, effective drugs can be validated more thoroughly by direct brain injection, electrophysiology, and behavioral methods. Significantly, validation of these screening approaches in a *D. melanogaster* model of seizures, where the fly contains a mutant allele of the GABA_A receptor, using a panel of current AEDs has already been performed, demonstrating the potential effectiveness of this model for high-throughput AED discovery (Stilwell et al., 2006).

7. Cognitive/Psychosis/Affective Disorders. Disorders of the CNS that influence affect and cognition are complex multifactorial diseases involving genetics and environmental factors. Traditional animal models of schizophrenia and depression used in the drug discovery process are problematic because they do not model the disease state in humans, they model only certain behavioral and neurochemical aspects (Nestler and Hyman, 2010). For example, traditional models of schizophrenia employ blockade of the behavioral effects of dopaminergic agonists such as apomorphine or amphetamine, as well as short-term administration of drugs whose effects

are thought to resemble psychosis, such as phencyclidine. Models of depression use forced swimming and tail suspension to identify agents able to prolong activity. Although drugs that are effective in these animal models have some efficacy in the clinic, precise therapeutic mechanisms of action remain largely unknown (i.e., atypical antipsychotics and selective serotonin-reuptake inhibitor antidepressants). There is currently a need for better animal models, as well as more effective therapeutics (Geyer, 2008; Nestler and Hyman, 2010).

Cognitive and affective disorders are generally regarded to involve disruption of key neurotransmitter systems, including dopamine, serotonin, and glutamate. Significantly, the fly CNS uses the same neurotransmitter systems to mediate many behaviors conserved with mammals, including humans. Because of this conserved neurochemistry, *D. melanogaster* can play an important role in the drug development process for CNS therapeutics. At present, the fly may be most valuable in target discovery experiments. Components of each of the neurotransmitter systems underlying particular behaviors in the fly, identified either through traditional or whole-genome analysis methods, may represent homologs of “druggable” targets in humans. One method of target discovery that holds promise is to express homologs of human genes linked to psychiatric diseases such as schizophrenia in fly brains to produce abnormal behaviors, as has been done for DISC-1, and to then perform genetic screens to identify modifiers whose human homologs may represent “druggable” targets (Sawamura et al., 2008; Furukubo-Tokunaga, 2009). In addition, the fly will be useful in post-HTS validation studies to rapidly and cost-effectively test the efficacy of compounds to block or inhibit behaviors mediated by these neurotransmitters in a whole-animal model. There are a number of behaviors and behavioral assays designed to assess function of these neurotransmitters and their receptors relevant to human neuropsychiatric disorders (Table 3).

Related to cognition and cognitive disorders is the process of learning and memory. The study of learning and memory in the fly has a long and rich history (Quinn et al., 1974; Tully and Quinn, 1985). Indeed, many of the molecular mechanisms underlying learning and mem-

TABLE 3
Neurotransmitter-related behaviors

NT/Receptor	CNS-Related Behavior	Reference
Serotonin	Feeding, aggression, courtship, sleep, learning and memory	Dierick and Greenspan, 2007; Sitaraman et al., 2008; Alekseyenko et al., 2010; Neckameyer, 2010
5-HT _{1A} -like	Aggression, sleep, learning and memory	Yuan et al., 2005; Johnson et al., 2008, 2009
5-HT ₂	Circadian, aggression, visual processing	Nichols and Sanders-Bush, 2002; Nichols, 2007; Johnson et al., 2008
5-HT ₇	Learning and memory, courtship and mating	Johnson et al. 2010; C. D. Nichols, unpublished data
Dopamine	Locomotor activity, arousal, circadian	Foltenyi et al., 2005; Johnson et al., 2010
D1	Learning and memory, prepulse inhibition	Lebestky et al., 2009; Waddell, 2010
D2	Locomotor activity, arousal	Draper et al., 2007
Glutamate	Social interaction, learning and memory	Grosjean et al., 2008
GABA	Sleep, circadian, learning and memory	Chung et al., 2009; Hamasaka et al., 2005; Davis, 2005
Acetylcholine	Learning and memory, circadian	Gu and O'Dowd, 2006; Hamasaka et al., 2007

ory in mammalian systems were first elucidated in the fly. The fly has short- and long-term memory involving acquisition, consolidation, and recall (Margulies et al., 2005). Sophisticated learning and memory assays have been developed to examine olfactory, appetitive, and place conditioning using both aversive and rewarding conditioned stimulus protocols (Davis, 2005; Sitaraman et al., 2008; Krashes et al., 2009). Neurotransmitters critical for proper learning and memory include dopamine, acetylcholine, GABA, serotonin, and glutamate (Gu and O'Dowd, 2006; Liu et al., 2007; Wu et al., 2007; Sitaraman et al., 2008; Waddell, 2010). Aside from the use of *D. melanogaster* for target discovery in basic science studies, flies can be potentially used in primary or validation studies for the identification of "cognitive enhancers" to be used as therapeutics for diseases that impair learning and memory or even age-related decline in learning and memory (Scott et al., 2002).

Although the fly brain is complex, composed of distinct neuropil with functional specialization and many conserved neurotransmitters and advanced behaviors as described above, it is not a human brain, and there are obviously significant differences. Therefore, certain features of the system need to be considered when designing screens and interpreting data. First and foremost, the fly brain is not capable of producing higher order cognitive behaviors that are associated with thought, affect, and other features that give can rise to uniquely human neuropsychiatric disorders. The fly may be most informative in elucidating molecular and genetic mechanisms and in small-molecule discovery for therapeutics relevant to specific behaviors that are associated with neuropsychiatric disorders (e.g., aberrant aggression, sleep, memory) rather than serve as a holistic model for disorders. Whereas the fly has most of the neurotransmitters found in mammals, there are key differences. For example, the fly does not have an adrenergic system and contains neither epinephrine nor norepinephrine nor the α and β adrenergic receptors. Instead, the fly uses octopamine, a trace amine in humans, as a major neurotransmitter that roughly performs similar physiological functions as the mammalian adrenergic neurotransmitter system (Evans and Maqueira, 2005). Furthermore, the fly does not have the full complement of receptors that mammals have for each conserved neurotransmitter. For example, there are six families of GPCR serotonin receptors in mammals and only three in flies, and five dopamine receptor families in mammals but only two in flies (for review, see Nichols, 2006). Moreover, the neurotransmitters themselves can be used differently than in mammals to regulate behaviors. In mammals, the primary excitatory neurotransmitter in the brain is glutamate, and at the neuromuscular junction, it is acetylcholine, but in the fly, the role of these two neurotransmitters are reversed (for review, see Nichols, 2006).

B. Cancer

In the past, cancer research has been conducted almost exclusively in mammalian-based systems ranging from tissue culture to whole-animal studies. Recently, however, the fly has been increasingly used as a model system. Perhaps one of the greatest contributions of the fly to the study of cancer biology was the elucidation of the Ras signal transduction cascade more than 20 years ago in the fly visual system (Simon et al., 1991; Olivier et al., 1993; Nagaraj and Banerjee, 2004). Each of the major components of this pathway have been found to be conserved in mammalian cells. At a fundamental level, cancer can be thought of as a misregulation of signaling events within a cell that leads to abnormal growth and proliferation. Depending on the type and nature of the cancer, the underlying mechanisms of the abnormal proliferation are varied and often remain elusive. Therefore, potential therapeutics are likely to be dependent on a detailed understanding of individual types of cancers. A so-called "magic bullet" effective against all or many types of cancer may still be possible, however, with further research into the nature of abnormal cell proliferation.

The majority of cancers in humans are derived from epithelial cells (Christofori and Semb, 1999), making these types of tumors significant targets for therapeutics. Accordingly, there are a number of fly models being developed to study epithelial cell-derived cancers that could and are being translated to a discovery platform. These models include not only proliferative phenotypes but metastatic and invasive ones as well. The challenge here is to develop effective screening paradigms that are able to identify agents able to prevent or inhibit proliferation and metastasis. One effective strategy has been to misexpress either the *D. melanogaster* version of a human signaling molecule linked to tumors, or the human protein itself, in the eye of the fly. The repeating "crystalline" nature of the eye make it highly susceptible to even slight perturbations in development, which usually manifest as a rough or disorganized phenotype easily scored by simple observation (Cagan and Ready, 1989). For example, Cagan and colleagues (Vidal and Cagan, 2006) misexpressed the fly homolog for the Ret receptor tyrosine kinase (implicated in human multiple endocrine neoplasia type 2), dRet, in a constitutively active form and produced a rough eye phenotype. They used this fly to both perform modifier screens to identify interacting factors as well as to validate efficacy of a small molecule inhibitor of Ret in vivo (Vidal and Cagan, 2006). Additional epithelial models using morphological changes in adult structural phenotypes have also been developed for discovery of molecules targeting the EGF receptor/ras pathway (Aritakula and Ramasamy, 2008), and E-cadherin (Pereira et al., 2006).

Alternative approaches have involved higher throughput strategies using larva and pupae. For example, one exciting model is a high-throughput platform examining

pupal viability as a measure of tumor suppression. In this model, invasive tumors and ultimately cell death at the pupal phase are produced from the expression of both a constitutively active form of Ras and a mutant of the tumor suppressor scribble together in imaginal discs (Pagliarini and Xu, 2003; Humbert et al., 2008; Wu et al., 2010). It is noteworthy that these flies were also engineered so that the tumors express the marker protein GFP, allowing for visual quantification of tumor size and metastasis. Assays are conducted in 96-well plates with a small number of larva seeded per well, with drug present in the media. After 5 days, a sucrose solution is added to the wells, and the dead larvae float to the top, where the GFP intensity, as a measure of tumor growth, can be measured by microscopy.

Another high throughput screening system relies upon flies with gain of function *raf* or a dominant negative allele of Notch, that each exhibit abnormal cell growth of midgut epithelium as a model for asymmetric stem cell division related cancers (Micchelli and Perri- mon, 2006; Januschke and Gonzalez, 2008). For this assay, *raf* or Notch mutant flies that express luciferase in gut epithelial cells are maintained on 96-well plates on media containing test drug, and then homogenized and assayed for luciferase activity as a measure of abnormal proliferation.

Additional opportunities for discovery lie in other types of cancers, including those derived from blood cells. Much work has been performed demonstrating conservation in blood cell development between flies and humans, including the study of *lozenge/Runt*-related transcription factors (Braun and Woollard, 2009) and JAK/STAT signaling (Bina et al., 2010) in hematopoietic cells; however, high-throughput assays for therapeutic discovery relevant to blood cancers such as leukemia remain to be developed. Nevertheless, there are limitations of the fly in cancer research. Whereas fundamental molecular mechanisms underlying tumorigenesis and metastasis can probably be efficiently probed in *D. melanogaster*, the fly is not able to model many types of tumors that are common in humans, such as those related to specific tissues (e.g., prostate, ovarian, or breast cancer).

C. Cardiovascular

Cardiovascular disease (CD) and related illnesses are the leading cause of death in the United States, and therefore a highly desirable area for development of new and more effective therapeutics. Recent work has indicated that the fly can be used successfully in the discovery process for CD. A key consideration to keep in mind is that cardiovascular diseases are for the most part complex multifactorial disorders that involve heredity as well as environmental factors, and that whereas certain aspects of CD can be modeled in the fly to yield informative results, the inherently complex nature of the cardiovascular system in humans presents certain

limitations in the fly for accurate modeling. For example, the fly heart has only one cardiac chamber and has no coronary arteries.

Fly heart development depends on a set of genes conserved up through mammals (Bryantsev and Cripps, 2009; Reim and Frasch, 2010), and sophisticated tools have been developed, including tomography, to allow its function to be probed in detail (Choma et al., 2006; Null et al., 2008; Bradu et al., 2009). Various forms of dysfunction that include structural defects, arrhythmias, and cardiomyopathies are known to occur in natural populations of flies (Ocorr et al., 2007c). Many of these effects can be age-related, and even result in cardiac failure in the fly (Ocorr et al., 2007a,b). Together, these aspects of the fly heart and its function indicate that the fly can be a valid model for the study of aspects of mammalian CD and an important tool in the process to discover new therapeutics (Wolf and Rockman, 2008; Wu and Sato, 2008; Akasaka and Ocorr, 2009). Significantly, the beating fly heart can be observed through a traditional dissection microscope for analysis. An excellent resource for protocols on visualization, dissection, and electrophysiological recording from larva heart is a publication from Robin Cooper and the accompanying video tutorial (Cooper et al., 2009). Using these methods, it is possible to easily examine the effects of pharmacological agents on heart function (Dasari and Cooper, 2006; Dasari et al., 2007; Neckameyer et al., 2007). Additional tools to facilitate examination of the heart include GAL4 drivers that can be used to express GFP in the heart, allowing for real-time observation of function with conventional epifluorescence or confocal microscopy (Wu and Sato, 2008; Alayari et al., 2009; Vogler and Ocorr, 2009).

So where does the fly fit in the overall scheme of the discovery process for CD? One important role is in the discovery of new targets through genetic methods to identify components crucial for heart function (Kim et al., 2010; Neely et al., 2010) for which subsequent traditional small-molecule discovery can then be performed against. There is also a role in the validation process of positive hits from more traditional screens to assess the actions of particular drugs on cardiac function using low-throughput methods (Akasaka and Ocorr, 2009). Given the recent advances in genetic and imaging tools available to examine fly heart function, it is hoped that higher throughput methods will be soon developed enabling this powerful model to be used for small molecule discovery.

D. Inflammation / Infectious Disease

D. melanogaster have a very sophisticated immune response that current research demonstrates is highly relevant to the understanding of human inflammatory conditions. Flies are constantly exposed to pathogens within their environment, largely in the form of bacteria, both as larvae and as adults. In response to patho-

gen challenge, antimicrobial peptides are released through two primary pathways that involve evolutionarily conserved components, including Toll and Toll-like receptors, as well as nuclear factor- κ B, tumor necrosis factor- α , and JAK/STAT signaling. Whereas *D. melanogaster* has a sophisticated innate immune system, largely evolved to combat bacterial and fungal pathogens, the fly does not have an adaptive immune system. Therefore, a potentially significant limitation is that the fly is not an appropriate model for the study of antibody- and lymphocyte-dependent adaptive immune defenses. The following articles are recommended for more comprehensive reviews of the basic physiology of the inflammatory response and immune signaling in the fly: Kaupila et al. (2003), Ferrandon et al. (2007), Wu and Silverman (2007), and Hetru and Hoffmann (2009).

Although it is conceivable that multiple human inflammatory conditions can be modeled and used in the discovery process, the *D. melanogaster* model for asthma, which is the most common chronic inflammatory disease of the lung, is arguably the most advanced. The *D. melanogaster* respiratory system is the trachea, which consists of approximately 10,000 interconnected and branching tubules. Significantly, there are many conserved genes and regulatory components between trachea development in the fly and lung development in mammals (Liu et al., 2003; Horowitz and Simons, 2008). Airway epithelial cells form the trachea, and they are the first line of defense against airborne pathogens. Unlike mammalian airways, the *D. melanogaster* trachea is much simpler and consists of only one type of epithelial cell (Whitten, 1957; Horowitz and Simons, 2008). Because there is only one type of epithelial cell, it is essentially a cell culture model within an intact organism, and an immune response initiated from any one part of the tracheal system is identical to that initiated from another. Inflammatory responses in the trachea to pathogens include Toll, tumor necrosis factor- α , c-Jun NH₂-terminal kinase, and JAK/STAT signaling activity (Wagner et al., 2008).

Opportunities therefore exist to further develop and use *D. melanogaster* in the asthma therapeutic discovery process (Roeder et al., 2009). One area where the fly shows particular promise is in target discovery. A number of genetic tools are available, including GAL4 drivers that can drive transgene expression specifically in the trachea (Shiga and Tanaka-Matakatsu, 1996; Liu et al., 2003). One method for exploration is to use these strains to drive small interfering RNA elements in the trachea to selectively knockdown expression of genes whose human homologs are important for airway physiology and the development of asthma to produce an abnormal physiological phenotype (Roeder et al., 2009). Genes and proteins identified by forward genetics and modifier screens that rescue these mutant phenotypes represent starting points for traditional high-throughput small-molecule discovery for drugs that would ben-

eficially influence function of not only the fly protein but also the human protein. Another role in target discovery, although more indirect, would be to use the fly as a platform to validate novel genes and proteins identified from human whole-genome association and next-generation sequencing studies (Moffatt et al., 2007) for function in airway epithelial cells and the trachea. Both of these approaches to target discovery have the potential to identify and validate key components of airway function that represent "druggable" targets for asthma therapeutics.

E. Metabolic Disorders and Diabetes

Obesity and related disorders such as diabetes are a significant health problem in the United States. Two thirds of the adult population is overweight, and nearly 4% of the population has diabetes. Accordingly, this is an attractive area for drug discovery. Although the fly has not yet been used in the drug discovery process for this area, recent advances in the understanding of metabolic processes, glucose homeostasis, and endocrinology in the fly have poised *D. melanogaster* as a valid model relevant to human metabolic disorders and diabetes that can be used in the therapeutic discovery arena. Although the molecular mechanisms and signaling underlying metabolic processes are conserved to a degree, a potential limitation of the fly here is that the structures mediating these processes are quite different. For example, the fly does not have a pancreas that secretes insulin or a liver. Furthermore, unlike for mammals, it is not possible to feed flies a "Western diet" and have them become obese and develop metabolic syndrome.

In the fly, there are neurosecretory cells (Nässel and Winther, 2010) in the brain that secrete insulin, as well as additional secretory cells that secrete a glucagon analog that together exhibit physiological and genetic parallels to the vertebrate endocrine axis (Wang et al., 2007; Haselton and Fridell, 2010). Ablation of the adult insulin-secreting cells can lead to increased glucose levels in the hemolymph (the "blood" of the fly), increased circulating lipids, and resistance to starvation, among other phenotypes (Baker and Thummel, 2007; Haselton and Fridell, 2010). Fat cells and the fat body in *D. melanogaster* perform functions similar to those of the mammalian liver and are regulated by insulin through mechanisms conserved in mammalian systems in terms of metabolism and triglyceride and glycogen storage (DiAngelo and Birnbaum, 2009).

With respect to the use of flies as a model to study diabetes, flies express a homolog of the human sulfonylurea receptor that, along with the Ir potassium channel, forms an ATP-sensitive potassium channel to regulate release of certain fly hormones, including a fly hormone with glucagon-like function (adipokinetic hormone) (Kim and Rulifson, 2004). It is noteworthy that antidiabetic sulfonylurea drugs, including glyburide and tolbutamide, affect glucose homeostasis in the fly through interactions with the ATP-sensitive potassium channel

on neurosecretory cells (Nasonkin et al., 1999; Kim and Rulifson, 2004). It is also noteworthy that flies deficient in insulin production demonstrate a delay in development as well as small body size, both as larvae and as adults (Rulifson et al., 2002; Kaplan et al., 2008; Ruaud and Thummel, 2008). Body size is an easily scorable phenotyp and may be useful in the development of high throughput screens for identification of small molecules able to rescue insulin secretion-deficient mutants. Therefore, flies may potentially be useful in the discovery, screening, and validation phases for diabetes/metabolic disorder therapeutics. Therapeutic classes amenable for discovery may be limited, however. For example, sulfonyleurea drugs have efficacy in insulin-deficient flies, but other classes of therapeutics, such as metformin have not been demonstrated to be effective.

V. Successful Examples of *D. melanogaster* in the Drug Discovery Process

As briefly addressed above, there have been several published reports in which the fly has been used for both primary screens and secondary validation of biologically active compounds for therapeutic discovery for a wide range of human diseases, ranging from neurodegeneration to cancer. In this section, we will go into more detail with a few specific examples of successful screens and how the fly was employed, with a focus on discovery in the neurodegeneration arena.

Fragile X syndrome (FXS) is a neurodegenerative disease that has been successfully modeled in flies (Jin et al., 2007; Sofola et al., 2007). Deletion of the human fragile X mental retardation gene (*FMR1*) ortholog in *D. melanogaster* (*Fmr1*) recapitulates several phenotypes associated with fragile X syndrome in humans, including defects in synaptogenesis, courtship behavior, and spermatogenesis (Wan et al., 2000; Zhang et al., 2001; Dockendorff et al., 2002; Morales et al., 2002). In an attempt to identify potential therapeutics, this *D. melanogaster* model was used as a primary screening platform to probe a drug library of 2000 FDA approved compounds (Spectrum Collection compound library; MicroSource Discovery Systems, Gaylordsville, CT) (Chang et al., 2008). For this screen, Chang et al. (2008) followed a high-throughput approach. *Fmr1*($-/-$) mutant embryos were sorted using flow cytometry methods, with 12 embryos automatically dispensed into individual wells of 96-well plates, where each well contained a 40 mM concentration of an individual drug from the Spectrum library dissolved in the food substrate. To score rescue of the rate of lethality that occurs before puparium formation that is associated with deletion of the *Fmr1* gene, the percentage of live pupae and adults in each well was counted manually. Of the 2000 compounds tested in this assay, 61 were found to rescue at least some of the lethality associated with *Fmr1*($-/-$) flies, and the top 25% of these hits (15 of 61) were selected for further

validation based on their more robust effects. Follow-up validation studies included dose response experiments using the same assay, where 9 of these 15 compounds showed a dose-dependent effect for rescuing the lethality associated with the *Fmr1*($-/-$) flies. It is noteworthy that three of these were related to the GABA signaling pathway, which is a major pathway underlying many of the symptoms of FXS (Krogsgaard-Larsen et al., 2000; Reith et al., 2006; Chang et al., 2008). These hits were GABA itself, nipecotic acid (a GABA reuptake inhibitor), and creatinine (a potential activator of the GABA_A receptor). It is noteworthy that treatment with bicuculline (a GABA_A receptor antagonist) rescues the lower dendritic spine density associated with knockout of the *Fmr1* gene in the mouse model of FXS (Selby et al., 2007). It remains to be seen whether nipecotic acid or creatinine, or any of the other drugs identified from this fly screen, are effective in the mouse model. Nevertheless, this screen clearly demonstrates that *D. melanogaster* models of human diseases and assay systems are well suited for the drug discovery process. Significantly, multiple high-quality hits were identified from screening only 2000 small molecules, and these compounds can serve as potential candidate compounds (alone or in combination) for developing therapeutic interventions of fragile X syndrome, although they will need to be further validated in a mammalian model system before moving to human clinical trials.

In addition to serving as a primary screening platform, the fly can also be extremely valuable as a secondary validation screen subsequent to traditional in vitro HTS. Using the fly in this manner to rapidly narrow down larger collections of hits to smaller and higher quality collections of leads for subsequent medicinal chemistry optimization and testing in rodent models will likely save considerable resources compared with proceeding with all primary hits in expensive optimization and rodent experiments and will ultimately facilitate the overall discovery process.

For example, in an attempt to identify new therapeutics for SBMA, an in vitro fluorescence resonance energy transfer-based cellular aggregation assay was performed against a library of 2800 biologically active compounds (Annotated Compound Library, Brent R. Stockwell, Columbia University, New York, NY) that assessed the ability of a drug to inhibit polyglutamine protein aggregation in mammalian cells. In this screen, there were 739 positive hits that reduced protein aggregation by more than 30% (Pollitt et al., 2003). It is noteworthy that treatment with one of the strongest hits, 4-[(1*R*)-1-aminoethyl]-*N*-4-pyridinyl-*trans*-cyclohexanecarboxamide (Y-27632), could robustly suppress neurodegeneration in a *D. melanogaster* model of polyglutamine disease (Pollitt et al., 2003). None of the compounds identified, however, had yet been approved for clinical use by FDA (Pollitt et al., 2003). Encouraged by the results from this first screening effort, where some of the more effective drugs

had efficacy in fly models of polyglutamate disease, the same group subsequently undertook another screen and tested 4000 biologically active compounds that contained a large number of FDA-approved drugs in the same fluorescence resonance energy transfer-based assay (Desai et al., 2006). These compounds were obtained from three different sources that included the Annotated Compound Library (ACL) of biologically active small molecules of diverse structure (2800 compounds), a kinase inhibitor collection (300 compounds provided by Dr. Kevan Shokat, University of California at San Francisco, San Francisco, CA), and the National Institute of Neurological Disorders and Stroke Custom Collection of FDA-approved drugs and natural products (1040 compounds).

The ten positive hits from this screen were subsequently tested in a *D. melanogaster* model of Huntington's disease, a poly-Gln degenerative disease similar to SBMA. This involved scoring a progressive photoreceptor neurodegeneration phenotype caused by expression of expanded Huntingtin protein (htt Gln93) in *D. melanogaster* photoreceptor cells as the measure, where degeneration can be quantified by the pseudopupil technique (Franceschini and Kirschfeld, 1971; Steffan et al., 2001). The pseudopupil technique is a simple assay that involves decapitating a fly, using a drop of clear nail polish to mount the head on a glass slide, and then shining a light through the back of the head to observe the general organization of seven visible rhabdomeres (the membranous structures of the photoreceptor neurons that contain the opsins) in the retina across several ommatidium (the individual "unit" that insect eyes are composed of). Degeneration in fly eye models of neurodegeneration perturbs the overall structure of the retina, leading to a progressive loss of pseudopupil organization that correlates with the degree of degeneration. At least 200 pseudopupils were scored from treated and untreated groups and photographed with a dissecting microscope (Steffan et al., 2001). It must be emphasized that treating, preparing, and scoring 200 flies and their retinas requires far less effort and resources than treating, preparing, and scoring even only a fraction of this number of rodent retinas or brains. Significantly, three out of the five drugs found to be effective in alleviating neurodegeneration in the fly were already approved by the FDA (nadolol, fosfanol, and levonordefrin) (Desai et al., 2006). As such, these are high-quality hits, requiring far less resources to bring to market as repurposed therapeutics for neurodegeneration than completely novel drugs needing to go through extensive toxicology testing and early-phase clinical trials demonstrating safety. These three FDA-approved drugs are, unfortunately, associated with some toxicity and are not approved for long-term therapy that would be necessary for patients with HD. The results of these screens, however, can guide further drug development and medicinal chemistry efforts to identify or generate less toxic analogs

that may be useful for Huntington's disease therapeutics (Zhang et al., 2005; Desai et al., 2006). Although the five hits from this screen need to be further validated in rodent models of HD, these studies emphasize that secondary screening in flies is a practical method to follow to narrow larger collections of hits to smaller but higher quality collections to proceed with for further development.

In another successful example of *D. melanogaster* in the drug discovery process, the fly was used as an initial whole-animal validation platform of a lead compound immediately after traditional in vitro HTS. This strategy can serve as an informative, economical, and rapid final "flight check" of lead candidates before medicinal chemistry optimization and/or whole-animal rodent studies to identify potential red flags or issues that might preclude further costly steps in its development. For this, Apostol et al. (2003) initiated a large drug screen using a library of 16,000 compounds (Chembridge, San Diego, CA) to identify potential new drugs that can inhibit polyglutamine protein-mediated aggregation using yeast expressing htt-103Q-EGFP as a primary platform. Htt-103Q-EGFP is cytotoxic in yeast and cells expressing mutant huntingtin protein grow poorly (Meriin et al., 2002). The primary screen identified nine compounds that significantly increased the mutant yeast's growth. These chemicals were then tested in in vitro mammalian cell culture models, where four were found to reduce polyglutamine-mediated aggregation in PC12 cells as well as COS cells (Apostol et al., 2003; Zhang et al., 2005). These four compounds, as well as three structural analogs, were next tested for their ability to inhibit aggregation in mouse hippocampal slice culture. The slices were from the brains of R6/2 transgenic animals that ubiquitously express human htt exon 1 with >150 polyglutamine repeats (HD 150Q), and have several similar neurological and biochemical features conserved with HD (Mangiarini et al., 1996). One compound, C2-8, structurally analogous to one of the original four hits, was found to be very effective at reducing aggregation in the mutant hippocampal slices (Zhang et al., 2005). The next step in the development process was to test the efficacy of this drug in a whole-animal model. A fly model of HD was chosen for initial tests, where C2-8 was found to be very effective in suppressing neurodegeneration (Zhang et al., 2005). Based upon these results, such effective compounds can be moved forward for testing in rodent whole-animal models with added confidence for success. In this strategy, if lead candidates are not found to be effective or prove toxic in an unexpected manner, then they may have similar outcomes in rodent whole-animal models, and their advancement through the pipeline could be paused for further investigation before investing significant resources to move the drug to rodent model testing.

VI. Resources

There are several online resources available for *Drosophila* geneticists (fly pushers) that provide crucial information about available mutant alleles, RNAi knockdown lines, human disease homologs in *D. melanogaster*, and whole-genome sequences. The most useful and comprehensive internet-based resource for the fly community, and those considering using flies, is Flybase (<http://flybase.org/>). Flybase also provides links to other stock centers and to virtually every relevant *Drosophila* information web site available. Following the link to Flybase below are additional internet resources arranged in alphabetical order.

A. Flybase and Other Internet Resources

The primary source of genetic and genomic information on *D. melanogaster* can be obtained from Flybase, a comprehensive “one-stop shop” for information and data on *D. melanogaster*, and new *D. melanogaster* researchers are encouraged to become familiar with this web site. Flybase provides a comprehensive and integrated view of data obtained from the published scientific literature, sequence databanks, and large-scale data providers of *D. melanogaster* material resources (such as mutant stocks or cell lines). Also included at Flybase are information on genes, annotation, gene sequences, transgene constructs, and their insertions in experimental genomes, and references to the literature. Most of the other important *D. melanogaster*-related web sites are linked at Flybase, making it easier for fly researchers to easily obtain or reach much of the available information from one site.

- Flybase: <http://flybase.org/>.
- Berkeley *Drosophila* Genome Project (home of the fly genome project): <http://www.fruitfly.org>.
- *Drosophila* Interaction Database (CuraGen) (assembled gene and protein interaction data from a variety of sources): <http://www.droidb.org/DBdescription.jsp>.
- *Drosophila melanogaster* Exon Database (a database that contains information on *D. melanogaster* exons presented in a splicing graph form): <http://proline.bic.nus.edu.sg/dedb>.
- *Drosophila* Polymorphism Database (a collection of all the existing polymorphic sequences in the *Drosophila* genus): <http://dpdb.uab.es/DPDB/dpdb.asp>.
- *Drosophila* Population Genome Project (accurate and complete description of genomes of collections of individuals from populations of *D. melanogaster* worldwide): <http://www.dpgp.org>.
- *Drosophila* Species Genomes (a comparative database of *Drosophila* species): <http://insects.eugenesis.org/DroSpeGe/>.
- DrosDel *Drosophila* Isogenic Deficiency Kit (a collection of isogenic *Drosophila* strains containing deletions covering most of the genome): <http://www.drosdel.org.uk>.
- *Drosophila* Genetic Resource Center (Kyoto) (stock

center and information database based in Japan): <http://www.dgrc.kit.ac.jp/en/index.html>.

- *Drosophila* Genomics Resource Center (resource center that provides cDNA clones, vectors, and cell lines): <http://dgrc.cgb.indiana.edu>.
- East Asian Distribution Center (provides antisera to segmentation gene proteins): <http://www.nig.ac.jp/labs/DevGen/segmentation>.
- Flybrain (an online atlas and database of the *Drosophila* nervous system): <http://flybrain.neurobio.arizona.edu>.
- FlyChip (provides *D. melanogaster* microarrays and screening services): <http://www.flychip.org.uk>.
- FlyEx (a database of segmentation gene expression): <http://urchin.spbcas.ru/flyex>.
- FlyMine (an integrated database for *D. melanogaster* and *Anopheles* spp. genomics): <http://www.flymine.org>.
- FlyMove (images and movies of *D. melanogaster* development): <http://flymove.uni-muenster.de>.
- FlyPNS (a database specializing in the embryonic and larval peripheral nervous system): <http://www.normalesup.org/~vorgogoz/FlyPNS/page1.html>.
- FlySNP (high density genome-wide map/database of single nucleotide polymorphisms): <http://flysnp.imp.univie.ac.at>.
- FlyTrap (stock collection of random enhancer trap strains with associated expression patterns in the UK): <http://www.fly-trap.org>.
- FlyTrap (stock collection of gene trap GFP strains at Yale): <http://flytrap.med.yale.edu>.
- Gene Disruption Project P-Screen Database (searchable database of gene disruption strains): <http://flypush.imgen.bcm.tmc.edu/pscreen>.
- Homophila (human disease to *D. melanogaster* gene database): <http://superfly.ucsd.edu/homophila>.
- Interactive Fly (a large database of information regarding all aspects of fly development with links to other important resources): <http://www.sdbonline.org/fly/aimain/1aahome.htm>.
- WWW Virtual Library–*Drosophila* (a list of links to various *D. melanogaster* online resources): <http://www.ceolas.org/fly>.

B. Stocks and Reagents/Services

The *D. melanogaster* public stock centers are valuable resource for obtaining a variety of tools (mutant strains, RNAi strains, balancers, and deficiency kits) for research. Among these stock centers, the Bloomington *Drosophila* Stock Center at Indiana University is the largest commonly used stock center by fly geneticists.

- The Bloomington *Drosophila* Stock Center at Indiana University: <http://flystocks.bio.indiana.edu/>.

- Drosophila RNAi Screening Center: http://flyrnai.org/RNAi_index.html.
- Drosophila TILLING Project: <http://tilling.fhcrc.org/fly/>.
- Drosophila Affymetrix GeneChip Arrays: <http://www.affymetrix.com/>.
- Duke University Model System Genomics: <http://www.biology.duke.edu/model-system/FlyShop/index.html>.
- Exelixis Drosophila Stock Collection at Harvard Medical School: <http://drosophila.med.harvard.edu>.
- Fly stocks of National Institute of Genetics (NIG-FLY): <http://www.shigen.nig.ac.jp/fly/nigfly/index.jsp>.
- Gene Disruption Project Database: <http://flypush.imgen.bcm.tmc.edu/pscreen/>.
- Kyoto Institute of Technology, Japan: <http://kyotofly.kit.jp/cgi-bin/stocks/index.cgi>.
- Vienna Drosophila Resource Center (RNAi strain collection): <http://www.vdrc.org>.

1. *Injection/Transgenic Production.* Besides the public stock centers, there are several private companies that provide routine services for microinjection of DNA to generate transgenics, screening for fluorescent phenotypes, and balancing of transgenic flies (The BestGene Inc., Genetic Services Inc., and Rainbow Transgenic Flies Inc).

- Genetic Services: <http://www.geneticservices.com>.
- The BestGene: <http://www.thebestgene.com>.
- Rainbow Transgenic Flies: <http://www.rainbowgene.com/services.html>.

2. *Companies Performing Preclinical Screening in Flies.* There are several companies that have been using *D. melanogaster* as a primary platform for screening for therapeutics for human diseases and behaviors such as learning, cognition, neurodegenerative diseases, diabetes, and cancer.

- Aktogen (15 years of experience in performing *D. melanogaster* behavioral test for learning and memory, also actively involved in drug discovery of human CNS-related disorders): <http://www.aktogen.co.uk>.
- Brain-wave Discovery (provides contract-based services for drug discovery projects related to learning/cognition, neurodegeneration, and novel target analysis): <http://www.brainwave-discovery.com/index.htm>.
- En Vivo Pharmaceuticals (focused on discovering and developing drugs for CNS-related disorders, particularly Alzheimer's disease and schizophrenia): <http://www.envivopharma.com>.
- Genescent Corp (drug discovery for aging related disorders using *D. melanogaster* as a model system): <http://www.genescent.com/>.
- Medros Pharmaceuticals (drug screening for cancer

metastasis and diabetes-related disorders using *D. melanogaster*): <http://www.medrospharma.com>.

- Molecular Libraries Program: Pathways to Discover (this is an National Institutes of Health-sponsored program for discovering drugs through HTS that can modulate a given biological pathway or disease state): <http://mli.nih.gov/mli/mlpcn/>.

C. Conferences and Courses

1. *Conferences.* There are many scientific platforms where the fly researchers can present their data and discuss new ideas. The Annual Drosophila Research Conference is considered the largest international meeting for the Drosophila community. In general, most of the other genetic meetings organize separate sessions on animal models that allow fly researchers to present their work.

- American Society of Human Genetics: <http://www.ashg.org/2010meeting/>.
- Annual *Drosophila* Research Conference: <http://www.drosophila-conf.org/2011/>.
- European *Drosophila* Neurobiology Conference: <http://www.meeting.co.uk/conferecare/neurofly2010/>.
- Cold Spring Harbor Laboratory Neurobiology of Drosophila: <http://meetings.cshl.edu/meetings/dros09.shtml>.
- EMBO Conference Series: the molecular and developmental biology of *Drosophila*: <http://cwp.embo.org/cfs3-10-03/>.
- EMBO symposia: Functional Neurobiology in Mini-brains: from Flies to Robots and Back Again: <http://www.esf.org/activities/esf-conferences/details/2010/confdetail324.html>.
- London Fly Meeting (biennial): <http://www.lfm2010.net/>.
- Model organism to human biology: <http://www.mohb.org/2010/>.
- *Swiss Drosophila* meeting: http://www.unifr.ch/zoology/assets/files/rev_Program-2010.pdf.

2. *Courses.* There are few courses on basics of *D. melanogaster* genetics regularly offered by the Cold Spring Harbor Laboratory and the Wellcome foundation. These courses are offered to a limited number of selected candidates and often offer a scholarship to defer the cost of the course.

- Drosophila Genetics and Genomics: <http://www.wellcome.ac.uk/Education-resources/Courses-and-conferences/Advanced-Courses/Courses/WTX027650.htm>.
- Neurobiology of *Drosophila* – Cold Spring Harbor Laboratory, NY: <http://meetings.cshl.edu/courses/c-dros10.shtml>.

D. Useful Books for Drosophila Research

There are several books on Drosophila that can also be used for teaching basic fly genetics to new researchers.

Among these books, *Fly Pushing: The Theory and Practice of Drosophila Genetics* by R. J. Greenspan, is the most popular.

- Ashburner MA, Golic KG, and Hawley RS (2004) *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Ashburner M and Wright TR (1979) *The Genetics and Biology of Drosophila*. Academic Press, San Diego, CA.
- Bate M and Martinez AA (1993) *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Demerec M (1994) *Biology of Drosophila*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Echallier G (1997) *Drosophila Cells in Culture*. Academic Press, San Diego, CA.
- Greenspan RJ (1997) *Fly Pushing: the Theory and Practice of Drosophila Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Hartenstein V (1995) *Atlas of Drosophila Development*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
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E. Small-Molecule Libraries

Below are listed sources of small molecule libraries potentially suitable for *D. melanogaster* screens. These are provided by small- to medium-size pharmaceutical companies involved in drug development process for a variety of human diseases. These companies produce or provide diverse classes of chemicals and sell them individually or in collections to the researchers for further testing. Among these, MicroSource, Inc provides several useful drug libraries such as “The Spectrum Collection” (1280 FDA-approved drugs collection) that cover a broad range of biologically active and chemically diverse compounds. It is noteworthy that each of the drugs in the Spectrum collection are already FDA-approved, potentially reducing preclinical testing necessary for new therapeutic uses while repurposing the drug.

- MicroSource Inc. (A leading provider of synthetic compounds as well as FDA approved drugs and natural products in 96-well plate format): <http://www.msdiscovery.com/home.html>.
- ChemBridge Corporation (offers drug libraries with over 700,000 diverse compounds and target based compounds): <http://www.chembridge.com/products.html>.
- Prestwick Chemical Library (Offers contractual drug discovery services and provides several small molecular libraries with diverse classes of compounds): <http://www.prestwickchemical.fr/index.php?pa=26>.

- ActiMol (Provides predesigned chemical libraries of 100,000 compounds in micro plate and vial format): <http://www.actimol.com/>.
- Comgenex (in addition to providing a diverse classes of chemical libraries of 200,000 compounds, this company also provides technical expertise to bridge the gap from genomic/proteomics to novel drug targets): <http://www.rdchemicals.com/targeted-compound-libraries/comgenex.html>.
- Analyticon Discovery (provides purified compounds and semisynthetic compounds from natural products and the drug libraries, has over 25,000 compounds): <http://www.ac-discovery.com/index.php>.
- Enamine (one of the largest providers of screening compounds for HTS, building blocks, custom synthesis and molecular modeling): <http://www.enamine.net/>.
- Life Chemicals (this company has a collection of 736,000 drug-like compounds and can provide several small molecule libraries with diverse compounds for drug discovery): <http://www.enamine.net/>.
- TimTech (leading provider of synthetic organic and natural compounds, targeted libraries, building blocks, and custom synthesized compounds for drug screening): <http://www.timtec.net/>.
- Vitas-M Laboratory Ltd. (provider of large organic compounds for drug discovery): <http://www.vitasmlab.com/>.
- Zelinsky Institute Inc. (source of organic compound libraries for biological screening, provider of building blocks and custom synthesis): <http://www.zelinsky.com/>.

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