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Drosophila models pioneer a new approach to drug discovery for Parkinson's disease

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Despite the prevalence and severity of Parkinson's disease (PD), little is known about the molecular etiology of this disease, and preventative and disease-modifying therapies remain elusive. Recently, linkage studies have begun to identify single-gene mutations that are responsible for rare, heritable forms of PD, which offer an opportunity to gain insight into the molecular mechanisms of this disorder through the creation and analysis of appropriate animal models. One model system that is tractable for these studies is the fruit fly, *Drosophila melanogaster*. Analysis of several *Drosophila* models of PD has revealed some surprising insights into the pathogenesis of PD and begun to highlight potential treatment strategies.

Parkinson's disease (PD) is a common neurodegenerative disorder that is characterized by the loss of dopaminergic (DA) neurons in the midbrain and the accumulation of proteinaceous intraneuronal inclusions known as Lewy bodies. Substantial evidence indicates that environmental and genetic factors both contribute to the etiology of PD [1]. Although simple, monogenic forms of parkinsonism appear to be relatively rare. In the past several years, significant effort has been invested in the identification and functional analysis of these genes in the hope of providing insight into the mechanisms responsible for more common, sporadic forms of PD [2] (Box 1). At present, linkage studies have identified nine loci that are responsible for simple Mendelian forms of PD, of which six have been cloned (Table 1).

Although the identification of genes that are responsible for heritable forms of PD has the potential to provide insight into the mechanisms of PD, and the interplay between genetics and environment in this disorder, we know very little about the biological functions of the genes that have been identified and how their mutational alteration results in neuronal death. One promising approach to this problem involves the use of classical genetic analysis in the fruit fly, *Drosophila melanogaster*. *Drosophila* has a complex nervous system that consists of ~100,000 neurons, and includes a subset of ~200 neurons that contain the neurotransmitter DA (Figure 1). Although the anatomy of the fly brain and the distribution of DA neurons in the central nervous system of *Drosophila* differs from that of vertebrate brains, many fundamental cellular and molecular biological features of neuronal development and function are conserved between vertebrates and invertebrates. This conservation makes *Drosophila* a powerful system for basic studies of neuronal development and function and, more recently, studies of neuronal dysfunction (Box 2). Furthermore, the completion of both human and *Drosophila* genome-sequencing projects has also revealed that a large fraction of human genes, including many involved in disease [3], have highly conserved counterparts in *Drosophila*. In particular, the *Drosophila* genome encodes homologs of five of the six PD-related genes that have been identified (Table 1).

Several vertebrate model systems offer a powerful collection of molecular and genetic tools to either perturb the functions of specific genes or to introduce foreign genes for further analysis. However, an important advantage of using *Drosophila* to understand human disease is the ability to conduct genome-wide genetic screens for mutations in other genes that modulate the phenotype associated with a disease model. This approach has the potential to identify genetic pathways that cause the disease, as well as those that can influence it, and does not require *a priori* knowledge of the function of the disease gene. The power of such screening approaches cannot be overstated: the human counterparts of

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1359-6446/06/$ – see front matter PII: S1359-6446(05)03693-7

suppressors identified from screens using *Drosophila* define potential targets for therapeutic intervention. Primarily, it is the feasibility of conducting such high-throughput screens that sets *Drosophila* apart from vertebrate models of disease. However, before performing these genetic screens, robust disease models must be established. The current *Drosophila* models of PD are discussed in the following sections.

The α-synuclein transgenic *Drosophila* model of PD: generation and characterization

The first gene shown to be associated with a heritable form of PD, *α-synuclein*, was also the first of the PD-related genes to be studied in *Drosophila*. Although mutations in the *α-synuclein* gene appear to be an extremely rare cause of PD, the finding that α-synuclein is a component of the Lewy body inclusions in patients with the sporadic form of the disease indicates that this factor might be a causative agent in most forms of PD [4,5]. Thus, insight gleaned from studies to uncover the mechanism by which α-synuclein induces neuronal loss might lead to the development of treatment strategies that will impact most cases of PD.

Whereas most of the human genes that are implicated in PD have counterparts in *Drosophila* (Table 1), there appears to be no *Drosophila* ortholog of the *α-synuclein* gene. However, the findings that both missense mutations and increased dosage of *α-synuclein* confer dominant forms of parkinsonism, indicate that the death of DA neurons in these heritable forms of PD result from the expression of either aberrant forms or concentrations of this protein rather than loss-of-function. To test this hypothesis, Feany and Bender [6] have generated transgenic flies that express wild-type and mutant forms of human α-synuclein throughout the brain. The heads from α-synuclein-expressing flies were sectioned and stained for tyrosine hydroxylase (TH) to assess the integrity of DA neurons. This analysis reveals progressive loss of neuronal integrity that is restricted to the PPM1/2 cluster (also called the dorsomedial cluster) of DA neurons (Figure 1), which is consistent with the hypothesis that α-synuclein is cytotoxic. Similar degrees of toxicity are detected following expression of wild-type α-synuclein and the A30P and A53T mutant forms. These toxic effects appear to be relatively specific to DA neurons in the central nervous system because the number of neurons that contain 5-hydroxytryptamine and gross brain morphology are unaffected in the *α-synuclein* transgenic flies [6]. However, expression of α-synuclein in the compound eye of *Drosophila* causes a retinal-degeneration phenotype, which indicates that α-synuclein can be cytotoxic in cell types other than DA neurons.

Neuronal expression of α-synuclein also results in the appearance of protein aggregates that crudely resemble the Lewy bodies in post-mortem tissue from idiopathic PD patients and is accompanied by an age-dependent locomotor (climbing) defect [6]. The appearance of this motor defect parallels the loss of DA neurons and the onset of aggregate formation, which indicates a mechanistic connection between aggregate formation, DA neuron loss and motor dysfunction. This initial work provides the foundation for subsequent studies of α-synuclein pathogenesis in *Drosophila*.

BOX 1

Potential mechanisms of Parkinson's disease pathogenesis.

Although the etiology of PD is understood poorly, there is evidence for several distinct neuropathological pathways [42]. For example, epidemiological studies have identified several environmental neurotoxins such as MPTP, paraquat and rotenone, that adversely affect mitochondrial function and increase oxidative stress. Genetic studies of mutations in PINK1, a putative mitochondrial protein, and DJ-1, a putative oxidative-stress-response component, provide further support for these mechanisms. Furthermore, human post-mortem tissue shows evidence of oxidative stress and mitochondrial dysfunction. By contrast, Lewy body inclusions in sporadic PD have led to the hypothesis that aberrant protein turnover is central to pathogenesis. This is supported by findings that mutations in parkin and Uch-L1, which both have a putative role in protein degradation, and α-synuclein, a prominent component of Lewy bodies, cause inherited forms of parkinsonism. Together, these findings indicate strongly that the mechanisms involved in rare, monogenic forms of PD are related to the more common, sporadic PD. Studies that target these genes in animal models offer the potential to reveal possible mechanisms and to test strategies for treating PD.

Applications of the *Drosophila* α-synuclein model

The coincidence of neuronal loss and the formation of α-synuclein-positive aggregates in α-synuclein transgenic flies has prompted several studies of the possible involvement of aggregate formation in the loss of DA neurons. Because previous work indicates that polyglutamine-containing proteins also form intracellular aggregates in *Drosophila*, and that polyglutamine pathology is attenuated by overexpression the chaperone HSP70 [7], Auluck *et al.* [8] have tested whether overexpresssion of HSP70 also alleviates α-synuclein toxicity. This study demonstrates that overexpression of HSP70 abrogates the α-synuclein-induced loss of TH-positive neurons without influencing the appearance of Lewy body-like aggregates. Furthermore, feeding *α-synuclein* transgenic flies with chaperone-inducing geldanamycin phenocopies the protective effect of HSP70 expression [9]. These results indicate that large, α-synuclein-containing aggregates are not sufficient for neuronal

TABLE 1

Putative function of human genes linked to PD and their fly homologs

PD locus	Gene or protein	Mode of inheritance	Fly homolog CG#	Fly homolog(s) identity, similarity	Putative function
PARK1	SNCA/α-synuclein	AD	No homolog	No homolog	Synaptic plasticity?
PARK5	UCH-L1	AD(?)	Uch/CG4265	45%, 66%	Ubiquitin hydrolase/ligase
PARK2	Parkin	AR	parkin/CG10523	42%, 59%	E3 ubiquitin-protien ligase
PARK7	DJ-1	AR	DJ-1a/CG6646, DJ-1b/CG1349	56%, 70% 52%, 69%	Oxidative stress sensor?
PARK6	PINK1	AR	CG4523	32%, 50%	Mitochondrial kinase
PARK8	Dardarin/LRRK2	AD	CG5483	26%, 43%	Kinase

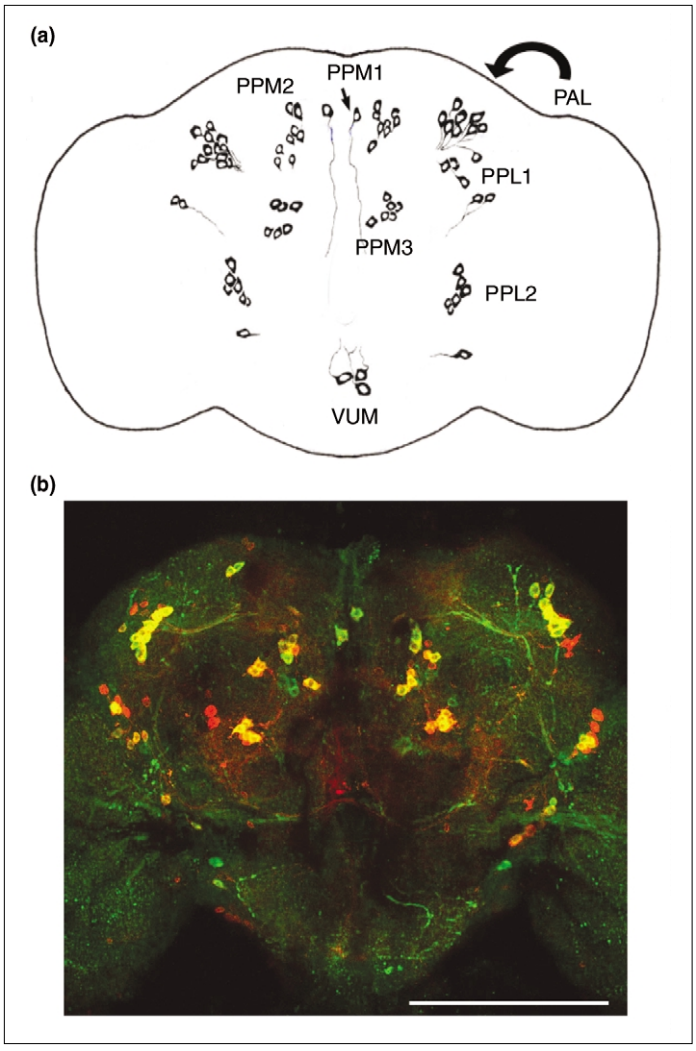

FIGURE 1

The dopaminergic system is well characterized in the adult nervous system. (a) Schematic representation of the distribution of DA neurons in the brain of adult *Drosophila*. DA neurons are grouped in small clusters arranged with bilateral symmetry. The PPM1/2 cluster is reported to be affected in the α-synuclein and DJ-1a models whereas the PPL1 cluster is affected by loss-of-function parkin mutations. **(b)** DA neurons revealed by confocal microscopy. Expression of an exogenous gene of choice, here a GFP-reporter gene (green), can be induced in most DA neurons in the fly brain. Co-staining with anti-TH antiserum (red) to highlight all DA neurons demonstrates significant, but not complete, overlap. A Z-series of optical planes is taken through the whole adult brain to allow accurate determination of all DA neurons. Abbreviations: PAL, protocerebral anterior lateral; PPM, protocerebral posterior medial; PPL, protocerebral posterior lateral; VUM, ventral unpaired medial. Scale bar = 100 μm.

loss, and that HSP70 might instead act on toxic, soluble, monomeric or oligomeric forms of α-synuclein. Alternatively, α-synuclein toxicity might result from titration of HSP70 into aggregates and the ensuing loss of HSP70 activity. Regardless of the mechanism by which HSP70 suppresses α-synuclein toxicity, the finding that feeding flies geldanamycin suppresses the loss of TH-positive cells indicates a possible treatment strategy for PD.

To explore further the relationship between the formation of α-synuclein aggregates and DA neuron loss, investigators have analyzed the effects of overexpressing the E3 ubiquitin-protein ligase parkin in the *α-synuclein* model. Loss-of-function mutations of the *parkin* gene in humans result in an early-onset form of autosomal recessive parkinsonism, and previous work shows that parkin binds to and ubiquitinates a glycosylated form of α-synuclein *in vitro* [10,11]. Studies exploring the involvement of parkin in α-synuclein-aggregate formation demonstrate that parkin overexpression suppresses the formation of α-synuclein-containing aggregates, but no affect on the overall abundance of α-synuclein protein is detected [12]. Further studies show that overexpression of *parkin* can suppress α-synuclein-induced retinal degeneration, climbing and DA neuron loss phenotypes [12,13]. These findings demonstrate that increased gene dosage of *parkin* can suppress α-synuclein pathogenesis. However, it is unclear whether α-synuclein is a direct target of parkin-mediated ubiquitination or whether parkin confers an indirect beneficial effect on the integrity of DA neurons. Moreover, the finding that overexpression of *parkin* ameliorates α-synuclein-aggregate formation appears to conflict with the observation that post-mortem analyses fail to detect Lewy bodies in most cases of parkinsonism caused by mutations of *parkin*. These studies do not provide direct evidence that the mechanism of pathogenesis associated with reduced parkin activity is mediated by α-synuclein.

Most recently, investigators have begun to explore the relationship between post-translational modifications of α-synuclein and aggregate formation and neuronal viability. Previous work indicates that α-synuclein is phosphorylated extensively at Ser129 in the brains of individuals with Lewy body pathology, but little or no Ser129 phosphorylation is seen in normal subjects [14]. Further work indicates that the Ser129 phosphorylation status of α-synuclein influences the propensity to form aggregates *in vitro* [14,15]. Interestingly, the phosphorylation of α-synuclein at Ser129 appears to occur in *Drosophila* [16]. To explore the consequence of phosphorylation of Ser129 on aggregate formation and neuronal integrity, Chen and Feany [17] have generated transgenic constructs in which Ser129 is mutated to either Ala (S129A), to prevent phosphorylation, or Asp (S129D), to mimic the phosphorylated state. They expressed these transgenic constructs in *Drosophila* and compared the extent and timing of pathology in these lines to flies that express wild-type α-synuclein. Expression of the S129D construct accelerates the onset of loss of TH-positive neurons, increases the magnitude of retinal degeneration and reduces the amount of α-synuclein in aggregates relative to wild-type α-synuclein. By contrast, expression of the S129A construct reduces the loss of TH-positive neurons and increases the total load of aggregated α-synuclein protein relative to wild-type α-synuclein. These results indicate that phosphorylation of Ser129 maintains α-synuclein in a non-aggregated, more toxic conformation. Furthermore, an increase in α-synuclein aggregates correlates with decreased cellular toxicity, which implies that Lewy body formation is a neuronal detoxification response.

These studies have several potential therapeutic implications. First, the evidence that the formation of protein inclusions is cytoprotective challenges current therapeutic strategies to prevent inclusion formation. Second, the finding that phosphorylation enhances the toxicity of α-synuclein indicates that the kinases responsible for phosphorylation represent therapeutic targets for small-molecule inhibitors. However, several important issues need to be addressed in order for the latter strategy to be viable. For example, although several protein kinases have been identified that phosporylate α-synuclein *in vitro* [15,18] and in flies, it is unclear

BOX 2

Animal models of Parkinson's disease: which one is best?

The mechanisms responsible for PD are being studied in several model organisms including mice, flies, worms and yeast. Each of these systems has its pros and cons. For example, because mouse and human biology are the most similar, findings in mice are potentially the most relevant to humans. However, the mouse system is by far the most costly and time-consuming. Although pharmacological studies in mice have contributed substantially to our understanding of the mechanisms responsible for PD, genetic models of PD in mice have largely failed to reproduce robust characteristics of PD. By contrast, simpler eukaryotic-model systems offer potentially cheaper, faster and more powerful genetic approaches. To date, efforts to create genetic models of PD in simple systems have resulted in robust phenotypes. However, the relevance of some of these findings to PD, particularly those from evolutionarily distant model organisms such as yeast, have been challenged. Perhaps the principle value of using multiple models of PD is that results in simpler systems might be used to improve the mouse models of PD. Ultimately, parallel findings in multiple model systems will provide the strongest arguments in favor of a particular mechanism of neurodegeneration.

which, if any, of these phosphorylates α-synuclein in the human brain. Perhaps more importantly, the main findings of Chen and Feany appear to conflict with previous work showing that phosphorylation of α-synuclein enhances the formation of inclusions in cell culture, and that highly phosphorylated α-synuclein is found primarily in Lewy bodies [14,19]. Further work is required to resolve these conflicts and determine whether inhibition of α-synuclein phosphorylation is neuroprotective.

Caveats of the *Drosophila* α-synuclein model

Although the remarkable degree with which the PD phenotypes are recapitulated in α-synuclein transgenic *Drosophila* make this an attractive model for studies of the pathogenesis of PD, it is important to point out that several key features of this disease model have not been reliably replicated. For example, ~50% of studies that examine locomotor ability in this model detect a climbing defect associated with α-synuclein expression [6,13,21], whereas others do not [8,20]. Although these conflicts are not reconciled easily, several possible explanations can be offered. Recent work indicates that dysfunction of DA neurons results in a hyperexcitable, 'startle' phenotype on vigorous mechanical disturbance of flies [22]. Thus, results might vary because vigorous tapping of vials induces a startle response that manifests as a climbing defect in response to DA neuron loss, whereas milder handling might fail to induce the startle response. Another possible source of variation in climbing behavior relates to a recent study that demonstrates that apparently homogenous fly populations consist of subpopulations with either 'high' or 'low' locomotor activity [23]. Failing to account for this phenomenon might result in situations in which more α-synuclein-expressing flies occur in the low-locomotor-activity state relative to the control population. Further work is required to resolve discrepancies in climbing behavior.

A more concerning challenge to the α-synuclein transgenic model was raised in a recent report by Pesah *et al.* [20], who failed to detect evidence of either loss of DA neurons or retinal degeneration, despite using the same wild-type α-synuclein transgenic lines reported by other investigators to cause these phenotypes. Expression of α-synuclein in *Drosophila parkin* mutants also fails to cause detectable loss of DA neurons [20]. These observations are supported by our unpublished work with these transgenic lines. Moreover, we also find that transgenic flies that express A30P α-synuclein fail to induce loss of TH-positive neurons (Table 2). A summary of the neuronal effects reported in the various *Drosophila* models of PD is shown in Table 2.

A recent study by Auluck *et al.* [24] strongly indicates that the conflicting results of DA neuron analysis in α-synuclein transgenic flies are likely to be explained by differences in the methodology used to analyze DA neurons. Whereas all the studies that document neuronal loss in α-synuclein-expressing flies utilize paraffin-embedded tissue and light microscopy techniques to visualize TH-positive neurons, Pesah *et al.* (and our own work) has used confocal microscopy of whole-mount brains to detect TH-positive neurons. Thus, the differences in the sensitivities of the detection methods might explain the conflicting results. Auluck *et al.* have addressed this possibility directly and demonstrate that the subpopulation of DA neurons that are reported to degenerate in α-synuclein-expressing flies is detected by confocal microscopy. Furthermore, they report that this particular cell population generally shows a less intense staining in α-synuclein transgenic flies than neurons in clusters unaffected by α-synuclein expression (supplementary data from [24]). These findings indicate that the loss of TH-staining that has been reported previously reflects reduced concentrations of TH rather than cell loss. Although the reduction in TH expression indicates that these cells are dysfunctional, it is unclear whether this phenotype reflects an early stage leading to the death of these neurons, as proposed by Auluck *et al.* [24]. Therefore, additional characterization of the α-synuclein transgenic model is warranted.

Mutational analysis of the *Drosophila parkin* gene

In contrast to the dominant, toxic, gain-of-function of α-synuclein mutations, at least three of the six genes that are known to be associated with heritable forms of PD appear to involve loss-of-function mutations (Table 1). Insight into the mechanisms by which these loss-of-function mutations cause PD requires detailed knowledge of the biological functions of the corresponding genes and the pathways that they regulate. One of the most powerful approaches to address these issues is classical genetic analysis in a simple model organism such as *Drosophila* to explore the biological functions of evolutionarily conserved homologs of these genes. This approach has been used recently to analyze the biological functions of *Drosophila parkin* and *DJ-1* homologs.

To explore the biological role of parkin, we and others have generated a series of mutations in the *Drosophila* ortholog of *parkin*, including deletion, nonsense and missense mutations. *Drosophila parkin*-null mutants are semi-viable and display reduced longevity, motor deficits and male sterility [25,26]. The motor deficit in *parkin* mutants is associated with a widespread apoptotic degeneration of muscle tissue, and the male sterility derives from a late defect in spermatogenesis. *Drosophila parkin*-null mutants also display degeneration of DA neurons in the PPL1 cluster, reduced TH-staining in the PPM1/2 cluster and significantly reduced DA content in the brain [27,28]. Importantly, the loss of DA neurons is detected by confocal microscopy; the more sensitive of the methods for

TABLE 2

Neuronal integrity in *Drosophila* models of PD

	Loss of TH-positive neurons?[a]				
Method	**Paraffin section/light microscopy**		**Whole-mount/confocal microscopy**		
Cluster	**PPL1**	**PPM1/2**	**PPL1**	**PPM1/2**	**Refs**
Model					
α-synuclein	No	Yes[b]	–	–	[6]
	Yes	Yes	–	–	[8]
	–	Yes	–	–	[9]
	–	Yes	No	No[c]	[24]
	–	Yes	–	–	[17]
	–	–	–	No	[20]
α-synA30P	–	–	–	No	(unpublished data)
parkin	No	No[d]	–	–	[25]
	–	–	–	No[e]	[26]
	–	No	–	–	[12]
	–	–	Yes	No	[28]
	–	No[d,f]	–	–	[27]
DJ-1a	–	–	No[f]	No[g]	[36]
	–	–	No	No	[37]
	–	Yes[f]	–	–	[40]
DJ-1b	–	–	No	No	[37]
	–	–	No	No	[38]

[a]Key: –, no data reported in this study.

[b]Variable neuronal loss.

[c]Partially reduced TH-staining without cell loss.

[d]Reduced TH-staining and cell shrinkage of TH-positive neurons.

[e]No alteration apparent in cellular morphology of TH-positive neurons.

[f]Reduced DA concentration in the brain.

[g]Suppression of age-dependent loss of TH-staining.

analyzing the integrity of DA neurons in *Drosophila*. Although the mechanism by which parkin influences DA neuronal integrity is unknown, recent work raises the possibility that it might act by suppressing activation of the c-Jun N-terminal kinase pathway [27].

Ultrastructural studies indicate that mitochondrial dysfunction is the earliest manifestation of muscle degeneration in *parkin* mutants, which indicates a role for parkin in mitochondrial integrity [25]. This conclusion is underscored further by the finding that late spermatids in *parkin* mutants show dramatic structural alterations in the Nebenkern, a mitochondrial derivative that is responsible for producing the energy for sperm motility. Whereas humans and mice with *parkin* mutations do not appear to manifest muscle and germline phenotypes, recent observations indicate that mitochondrial defects are a conserved feature in all organisms with *parkin* mutations [29,30] and that, normally, at least some parkin protein appears to localize to mitochondria [31]. These observations indicate that parkin might regulate mitochondrial integrity directly and that the mitochondrial defects observed in disparate species bearing *parkin* mutations might have a common biochemical basis.

In an ongoing effort to elucidate the mechanism underlying the pathology associated with loss-of-function of parkin in *Drosophila*, we recently conducted a genetic screen for mutations in genes that either enhance or suppress a *parkin* phenotype [32]. Ideally, we would like to conduct screens for suppressors of the loss of DA neurons, but this phenotype is not amenable to high-throughput analysis. Thus, we chose to use the partial lethality of *Drosophila parkin* mutants as a surrogate phenotype and screened for mutations in genes that either enhance or suppress lethality. The most potent enhancer of the *parkin*-recessive lethal phenotype recovered from our screen is a loss-of-function allele of the *glutathione S-transferase S1* (*GstS1*) gene. Members of the GST family of polypeptides are thought to act in cellular-detoxification pathways by catalyzing the covalent coupling of glutathione to several toxins, including the cellular products of reactive oxygen species [33].

In an initial effort to test the relevance of genetic factors that influence the viability of *parkin* mutants to the DA neuron loss phenotype, we explored the effect of altered GstS1 dosage on DA neuron integrity in *parkin* mutants [32]. The results of this analysis demonstrate that reducing the activity of GstS1 enhances neuron loss in *parkin* mutants whereas transgenic overexpression of GstS1 suppressed significantly the loss of DA neurons in *parkin* mutants [28]. The implications of these results are threefold. First, they demonstrate that the partial lethality of *parkin* mutants effectively serves as a surrogate phenotype for neuronal loss, and supports the validity of further screening. Second, our work indicates that several factors that have been implicated in parkin pathogenesis, such as α-synuclein and PaelR, for which there appear to be no *Drosophila* counterparts, are not obligate for DA neuron degeneration. Third, and most importantly, because several dietary components and

drugs induce the expression of glutathione *S*-transferase and glutathione (GSH) production in vertebrates [34], our findings indicate that these compounds should be tested as potential therapeutics for preventing PD.

Mutational analysis of the *Drosophila* DJ-1 gene family

Recently, *Drosophila* has also proved useful in dissecting the role of a third PD-related gene, *DJ-1*. Similar to *parkin*, loss-of-function mutations in *DJ-1,* which encodes a small protein of unknown function with homology to proteases, kinases and small heat shock proteins, result in parkinsonism in humans [35]. The *Drosophila* genome encodes two homologs of the human *DJ-1* gene, designated *DJ-1a* and *DJ-1b* (Table 1). *DJ-1b* appears to be expressed ubiquitously whereas expression of *DJ-1a* is restricted largely to testes [36–38]. To explore the biological roles of *DJ-1a* and *DJ-1b* we, and others, have used classic genetics and RNAi to perturb the functions of these genes [36–40]. The results of mutational analyses of the *DJ-1b* gene indicate that *DJ-1b* mutants are fully viable, fertile and display no obvious external/visible phenotypes and developmental abnormalities, and show no evidence of DA neuron loss [36–38]. However, two studies of *DJ-1b* mutants provide evidence that these mutants have striking sensitivity to chemical agents that induce oxidative stress, including paraquat and rotenone, chemicals linked epidemiologically to PD [37,38]. Moreover, treatment of WT flies with these oxidative-stress-inducing chemical agents results in a change in the electrophoretic mobility of DJ-1b, which indicates that DJ-1b is a direct target of an oxidative modification, as is vertebrate DJ-1 [37].

By contrast, work by Menzies *et al.* [36], using independently generated alleles of the *DJ-1b* gene, found that these mutants are less sensitive to exposure to paraquat than wild-type flies. Moreover, the authors report that *DJ-1b* mutants exhibit delayed age-dependent reduction of TH-staining in the brain. The authors ascribe these two phenotypes to a compensatory induction of *DJ-1a* expression in response to loss of *DJ-1b* function, and provide expression and transgenic data to support this. Although additional work is required to explain the discrepancies in studies of DJ-1b function in *Drosophila*, there are two likely explanations. First, the studies that report increased sensitivity of *DJ-1b* mutants to oxidative-stress agents use of definitive null alleles of the *DJ-1b* gene, but the alleles used by Menzies *et al.* might be hypomorphic in nature. Thus, the latter results might reflect a compensatory function of DJ-1a that is either only activated or detected in a *DJ-1b* hypomorph. Alternatively, the discordant results might be explained by differences in genetic background. Whereas our study of *DJ-1a* and *DJ-1b* involves comparisons with isogenic control flies, the work of Menzies *et al.* compares *DJ-1b* mutants with unrelated, wild-type lines. In support of this hypothesis, we observe significantly different chemical sensitivities in studies of genetically unrelated wild-type control strains (unpublished observations), thus, underscoring the importance of controlling for genetic background in such studies.

Genetic studies of the *Drosophila DJ-1a* gene have led to starkly contrasting results. Our mutational studies of *DJ-1a* indicate that *DJ-1a*-null mutants are fully viable with no demonstrable phenotype and chemical sensitivity [37]. Moreover, *DJ-1a:DJ-1b*-double mutants are also fully viable, have no obvious morphological or behavioral phenotypes (including an absence of detectable DA neuron loss) and exhibit a sensitivity to oxidative-stress agents that is identical to that of *DJ-1b* mutants alone [37]. By contrast, a genetic screen for mutations that enhance a phenotype of the tumor suppressor PTEN led to the recovery of a recessive lethal P element in the proximity of the *DJ-1a* gene, which indicates that *DJ-1a* is an essential gene and that DJ-1a negatively regulates PTEN [39]. Further support for these findings comes from a recent study by Yang *et al.* [40], who have used RNAi to target the *DJ-1a* gene. In this study, ubiquitous knockdown of the *DJ-1a* transcript results in larval lethality whereas targeted knockdown of *DJ-1a* in either the compound eye or in the nervous system results in photoreceptor-cell loss and progressive loss of TH-positive neurons in the PPM1/2 cluster (documented using the paraffin sectioning/light microscopy methodology), respectively. Cultured neurons from *DJ-1a* RNAi-treated animals also have an increased production of reactive oxygen species and increased sensitivity to oxidative stress inducing agents. In addition, Yang *et al.* also show that signaling through the phosphatidylinositol 3-kinase/Akt pathway suppresses the *DJ-1a* RNAi-induced eye and DA neuron phenotypes, possibly by reducing the production of reactive oxygen species.

Further work is required to resolve the discrepancies in studies of DJ-1 function in *Drosophila* but, despite the many individual conflicts, all studies of the DJ-1 gene family in *Drosophila* point to an important role in the management of oxidative stress. A major

TABLE 3

Key features and potential therapeutic strategies identified by analyzing *Drosophila* models of PD

Model	Associated phenotypes	Potential therapeutic
α-synuclein	Dysfunction of DA neurons[a]	Induce heat shock factors/chaperones (e.g. geldanomycin)
	Locomotor deficits[a]	Modulate the phosphorylation of α-synuclein
	Retinal degeneration[a]	
	Lewy body-like protein aggregates	
parkin	DA neuron loss	Induce phase II detoxifying enzymes (e.g. sulphoraphane)
	Mitochondrial pathology	Induce expression of antioxidant enzymes
	Locomotor deficits	
	Sensitivity to oxidative stress	
DJ-1	Sensitivity to oxidative stress	Induce expression of antioxidant enzymes
	Modification of DJ-1	

[a]Conflicting phenotypes are reported.

challenge is to discern between the myriad biological functions ascribed to this protein family to define the mechanism by which loss of DJ-1 function results in neuronal loss. Clues should emerge from the application of classical genetic methods to identify suppressors of the DJ-1 chemical-sensitivity phenotype.

Conclusions

The fruit fly *D. melanogaster* has been an invaluable model for basic studies of genetics and biology for nearly 100 years. Given the remarkable degree of genetic, molecular and cell-biological conservation between flies and mammals that has been revealed over the decades, *Drosophila* remains a valid model system in which to address novel biological questions, including those that are relevant to human health. Indeed, work aimed at an understanding of the genes involved in heritable forms of PD has already made significant contributions to our understanding of this debilitating disease. Moreover, these studies have begun to define small-molecule compounds that might impinge on the pathways implicated in PD (Table 3). Whereas these compounds offer real potential as therapeutic strategies, *Drosophila* models also lend themselves directly to unbiased, high-throughput screening of small-molecule libraries in the search for novel compounds that prevent pathogenesis. EnVivo Pharmaceuticals has taken such an approach by developing an automated platform for *in vivo* screening of compound libraries in several *Drosophila* models of neurodegenerative disease, and has begun to move several positive hits forward in the drug discovery process [41].

The *Drosophila* system has enormous potential in studies aimed at a mechanistic understanding of PD. PD-modeling in *Drosophila* is relatively new and the reagents and methodologies for conducting these studies are still evolving. In particular, future work should focus on defining the most appropriate methods for analyzing the integrity of DA neurons, and on resolving conflicts in some of the current *Drosophila* models of PD. It is also imperative that we have realistic expectations of these models. For example, at present the phenotypes of the loss-of-function models do not match precisely the phenotypes of humans with mutations in the corresponding genes. Although this might concern some, phenotypic differences that result from mutations in orthologous genes in different species often belie significant underlying conservation of molecular pathways. Indeed, the weight of evidence indicates that the phenotypes that are associated with the parkin and DJ-1 models in *Drosophila*, which include sensitivity to oxidative-stress agents and mitochondrial dysfunction, are directly relevant to the mechanisms implicated in PD. We have only just begun to tap the insight that modeling PD in *Drosophila* can provide: this will be fully realized only if we focus our efforts on understanding what the phenotypes of these models tell us and take full advantage of the power of genetics to lead us down unexpected paths.

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