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Review Article

Drosophila Models of Parkinson's Disease: Discovering Relevant Pathways and Novel Therapeutic Strategies

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Parkinson's disease (PD) is the second most common neurodegenerative disorder and is mainly characterized by the selective and progressive loss of dopaminergic neurons, accompanied by locomotor defects. Although most PD cases are sporadic, several genes are associated with rare familial forms of the disease. Analyses of their function have provided important insights into the disease process, demonstrating that three types of cellular defects are mainly involved in the formation and/or progression of PD: abnormal protein aggregation, oxidative damage, and mitochondrial dysfunction. These studies have been mainly performed in PD models created in mice, fruit flies, and worms. Among them, Drosophila has emerged as a very valuable model organism in the study of either toxin-induced or genetically linked PD. Indeed, many of the existing fly PD models exhibit key features of the disease and have been instrumental to discover pathways relevant for PD pathogenesis, which could facilitate the development of therapeutic strategies.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting more than 1% of the population over age 60. Clinically, it is characterized by locomotor defects such as muscle rigidity, bradykinesia, postural instability, and tremor. The principal neuropathology that gives rise to these motor defects is the progressive and selective loss of dopaminergic (DA) neurons in the Substantia nigra pars compacta, which causes a deficiency of brain dopamine content. Another pathological hallmark of this disorder is the presence of cytoplasmic inclusions in the surviving DA neurons called Lewy bodies (LBs), which are mainly composed of α-Synuclein and ubiquitin among other proteins [1, 2]. However, it has been shown that such structures are not present in some genetic forms of PD.

Although the majority of PD cases are sporadic and are probably caused by a combination of risk factors like the aging process, genetic propensity, and environmental exposures, few environmental triggers have so far been identified. Weak associations between PD and exposure to environmental toxins or herbicides and pesticides have been reported [2], and several toxin-induced PD models have been developed [3]. However, epidemiological studies have also demonstrated the contribution of genetic factors in the pathogenesis of PD. Indeed, during the last decade, several loci whose mutations are causative of rare familial forms of the disease have been identified, which account for 5%–10% of all PD cases. These genes include *α-synuclein*, *parkin*, ubiquitin C-Terminal hydrolase-1 (*UCHL-1*), *DJ-1*, phosphatase and tensin homolog (PTEN)-induced kinase 1 (*PINK1*), leucine-rich repeat kinase 2 (*LRRK2*), *Omi/HtrA2*, *ATP13A2,* and glucocerebrosidase (*GBA*) [4–14]. However, it is noteworthy to mention that the relevance of some of them to PD is currently under debate [15]. Despite this, studies of the function of PD-linked genes have provided important insights into PD pathogenesis and have demonstrated that three types of cellular defects are mainly involved in the formation and/or progression of the disease: abnormal protein aggregation, oxidative damage, and mitochondrial dysfunction [16]. Due to the limitations of human genetic analysis, most of these studies have been performed in

model organisms, including mice, fruit flies, and worms as well as in cell culture. Indeed, there are currently many cellular and animal models of PD either genetic or toxin-based. Cellular models can be easily used for molecular, biochemical, and pharmacological approaches, but they can lead to misinterpretation and artefacts. In contrast, animal models allow studying a cellular process in the context of a whole organism and are thus more reliable. Despite this, it is also remarkable that none of the existing PD animal models recapitulate all PD symptoms, including those developed in mice [17].

In such a scenario, the fruit fly Drosophila has emerged as a valuable model for studying mechanisms of human neurodegenerative diseases, including PD. Although fruit flies seem to be completely unrelated to humans, fundamental cellular processes as well as many genes and signalling pathways are conserved between both organisms. Moreover, most of the genes implicated in familial forms of the disease have at least one fly homolog [18]. In addition, flies are capable of performing complex motor behaviours such as walking, climbing, and flying and their brain is complex enough to make these behaviours relevant to humans. The availability of very potent genetic tools that are impractical in mammals, their rapid growth and reproduction, and the fact that it is cheap and easy to maintain in the laboratory are features that make Drosophila an ideal model system to address novel biological questions including those relevant to human health [19–21]. Indeed, studies of genes involved in familial PD as well as the development of toxin-based models of PD in Drosophila have made significant contributions to our understanding of the disease [15, 22, 23]. Here, we have attempted to provide a comprehensive review on existing Drosophila models of PD, which have revealed valuable insights into potential pathogenic mechanisms and have been used to target modifiers of PD pathology by genetic or pharmacological interference.

2. Toxin-Induced Models of PD in Drosophila

As indicated above, familial PD cases are extremely rare, which suggests that environmental factors or gene-environment interactions play a predominant role in the development of sporadic PD. For that reason, several studies have been performed to model PD-associated neuron loss by neurotoxin intoxication in animals, the most popular parkinsonian neurotoxins being 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat [3, 24]. In general, toxin-induced PD models do not recapitulate the process of progressive neuron loss and the protein aggregation in LBs, due to the acute nature of the neurotoxin treatment [15], but they have been useful to support the notion that alterations in mitochondrial biology are essential for the development of PD [25]. Indeed, mitochondria are central to the actions of the above-mentioned toxins, which preferentially injure DA neurons. In Drosophila, several studies have shown that pharmacological treatment could be used to model sporadic PD. First, chronic exposure to the pesticide rotenone, a mitochondrial complex I inhibitor, recapitulated key aspects of sporadic PD in Drosophila since it resulted in neurodegenerative and behavioural defects [26]. Indeed, rotenote-treated flies showed dose-dependent motor deficits quantified by a negative geotaxis test, which is commonly used to perform locomotor ability analyses in Drosophila, as well as selective loss of DA neurons in all the brain clusters. In a different study, paraquat exposure caused reduced lifespan in flies as well as movement disorders such as resting tremors, bradykinesia, rotational behaviours, and postural instability, which mirror PD symptoms. These complex set of locomotor phenotypes were overall quantified by a negative geotaxis test. The authors also demonstrated that such phenotypes were caused by selective loss of DA neuron clusters [27]. Thus, both studies robustly modelled environmental toxin-induced PD in Drosophila and provide useful tools for studying the mechanism of DA neurodegeneration. Drosophila models of MPTP- or 6-OHDA-induced Parkinsonism have not been established so far.

3. Drosophila Models of Familial PD

The discovery of several genes affected in familial forms of PD has provided a new tool for PD modelling. Indeed, many PD animal models have been generated based on gene mutations that are linked to the disease including Drosophila [15, 17, 19–21, 23, 28]. Although Drosophila PD models cannot recapitulate fully the phenotypic and pathologic features of human PD patients, loss of DA neurons and locomotor defects have been observed in most of them. Moreover, they have offered the advantage of identifying evolutionary conserved pathways and cellular processes relevant to PD pathogenesis.

Different approaches have been used to generate PD models in Drosophila. In some cases, no Drosophila orthologs of a specific PD-linked gene do exist. Then, the model is generated by misexpression of the human gene either in its wild-type or mutant form, which is usually achieved by using the GAL4/UAS system [29]. Widely used in Drosophila genetic studies, this system allows time- and tissue-specific misexpression of any gene of interest in flies. Alternatively, when an ortholog of the human gene is present in the Drosophila genome, loss-of-function (LOF)/knockdown alleles of the gene can be generated by different genetic techniques, including RNAi. Moreover, misexpression of the corresponding gene can also be carried out. In general, misexpression of either human or Drosophila PD-related genes is performed when the PD forms associated to them have a dominant inheritance. In that case, Drosophila PD models are established using GAL4 drivers specific of the nervous system or of other tissues, like eyes or wings, in which a possible phenotype can be easily identified without affecting fly survival. LOF/knockdown alleles are phenotypically characterized when the PD forms associated to the corresponding genes have a recessive inheritance. By using any of these strategies, several Drosophila PD models based on different PD-linked genes have been generated. Examples of phenotypes obtained in these models are shown in Figure 1.

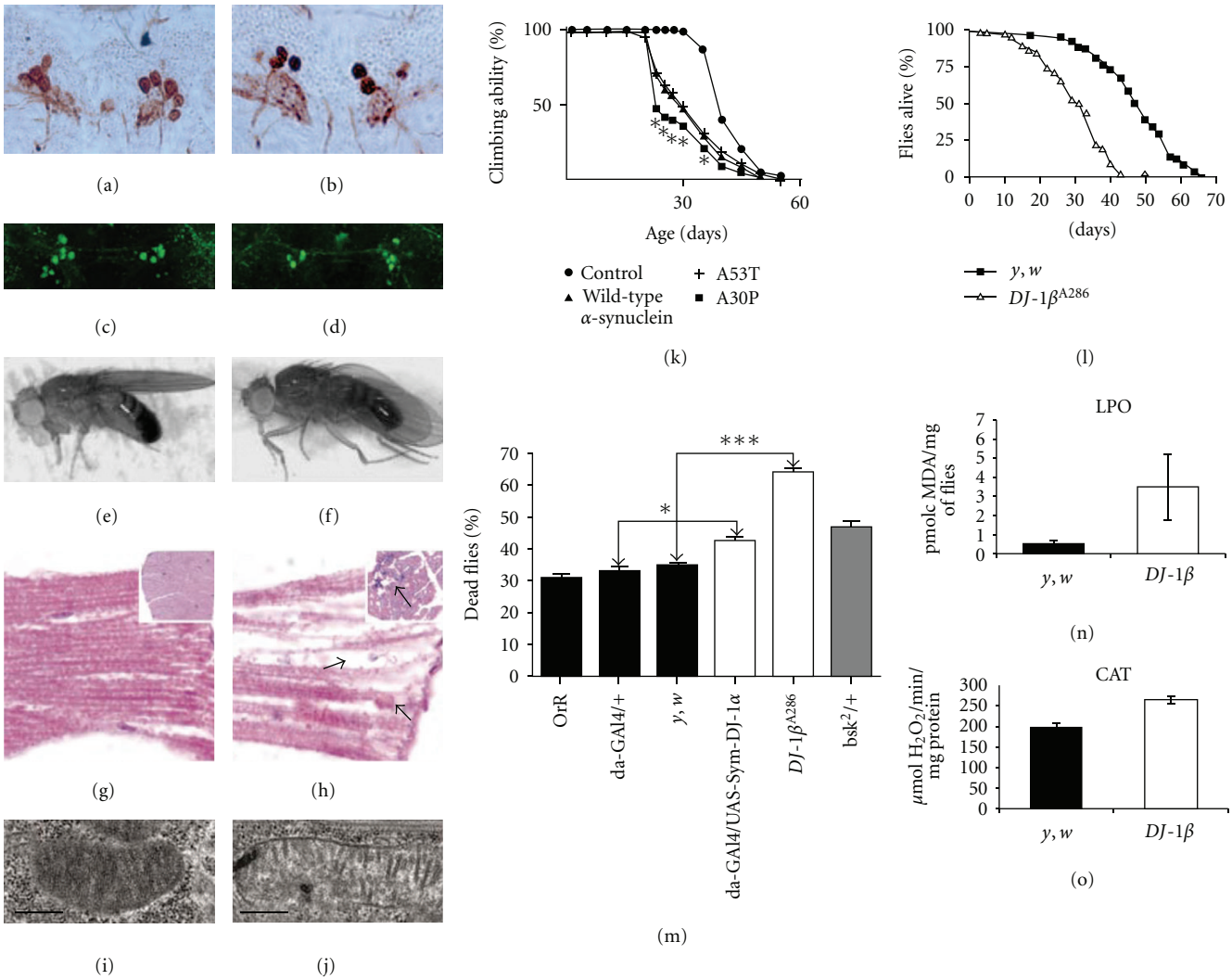

Figure 1: Representative phenotypes found in different Drosophila PD models. (a)–(d) DA neuron loss detected in Drosophila adult brains by immunostainings with anti-TH antibody, which specifically recognizes these neurons, in paraffin sections (a, b) or whole-mount brains (c, d). A reduction in the number of DA neurons is observed in both *Ddc-GAL4/DJ-1α RNAi* (b) [50] and *Ddc-GAL4/UAS-α*-Synuclein (d) [51] brains when compared to age-matched *Ddc-GAL4/+* controls (a, c). (e)–(j) Examples of phenotypes observed in *parkin* LOF mutants (f, h, j) compared to controls (e, g, i). They include downturned wings (f), muscle degeneration (h), and abnormal mitochondrial morphology (j) [52]. (k) Premature loss of climbing ability in transgenic flies expressing wild-type, A30P, and A53T mutant forms of α-Synuclein [34]. (l) Reduced lifespan of *DJ-1β* mutants compared to *y, w* control flies cultured under the same conditions. (m) Elevated sensitivity to paraquat stress in *DJ-1α* and *DJ-1β* mutant flies, represented by calculating the percentage of dead flies after feeding 15 mM for 18 h [53]. (n)–(o) Quantification of oxidative stress levels in 1-2-day-old *DJ-1β* mutants and age-matched *y, w* control flies. *DJ-1β* mutants show an increase in lipid peroxidation (LPO) product malondialdehyde (MDA) (n). Catalase (CAT) enzymatic activity is also increased (o) [54].

3.1. α-Synuclein. It encodes a small protein whose physiological function remains to be elucidated. However, mutations in the *α-synuclein* gene such as amino acid substitutions (A30P, E46K, and A53T), duplications, and triplications are causative of dominantly inherited forms of PD [4, 30–32]. Interestingly, α-Synuclein is one of the major structural components of LBs [33]. The first fly PD model was generated by overexpression of transgenes encoding either wild-type or mutant forms of human α-Synuclein in all Drosophila neurons since the Drosophila genome does not contain a clear *α-synuclein* homolog [34]. This resulted in an age-dependent and selective (complete or near complete) loss of DA neurons in the dorsomedial clusters (DMC) of the brain and formation of fibrillar α-Synuclein inclusions as well as a progressive loss of climbing ability, thus reproducing key PD features. Although several discrepancies regarding DA neuron loss upon *α-synuclein* overexpression were reported in subsequent studies [35, 36], associated to the different sensitivity of the methods used for DA neuron detection, recent analyses have confirmed that phenotype [37–39]. DA neurons were initially detected in paraffin-embedded brain sections stained with a specific marker (anti-Tyrosine hydroxylase (TH) antibody) (Figures 1(a) and 1(b)), but subsequent analyses were performed in whole-mount brain

preparations by confocal microscopy (Figures 1(c) and 1(d)). It has been proposed that while in paraffin-embedded sections only healthy DA neurons can be detected, some fluorescence is still observed in degenerating DA neurons. In any case, this fly model has been instrumental to decipher the neuropathological effects of the α-Synuclein protein as well as the regulation of aggregate formation. It has been demonstrated that inhibition of endoplasmic reticulum (ER)-Golgi trafficking and oxidative stress induction are major components of α-Synuclein-dependent toxicity [37–40]. Moreover, quantitative proteome analyses performed either on wild-type, A30P, or A53T α-Synuclein overexpressing flies at different disease stages revealed that deregulated proteins are primarily associated with membrane, endoplasmic reticulum, actin cytoskeleton, mitochondria, ribosome, cellular metabolism, and signalling [41–44]. Regarding α-Synuclein aggregation, overexpression of truncated forms of α-Synuclein in flies led to discover a central hydrophobic region of the protein which is essential for its aggregation as well as sequences C-terminal to residue 120 that have a more moderate role in influencing both aggregation and toxicity [45]. Moreover, several posttranslational modifications seem to regulate aggregation and toxicity of α-Synuclein. While phosphorylation of this protein at serine 129 is prominent in PD and influences α-Synuclein DA toxicity [46], phosphorylation at tyrosine 125 inhibits toxic oligomer formation and decreases with aging [47, 48]. These data suggest that α-Synuclein neurotoxicity in PD and related synucleinopathies may result from an imbalance between different C-phosphorylation events on the protein, regardless of the impact of such modifications on the normal function of α-Synuclein [48, 49].

3.2. Parkin. Mutations in the *parkin* gene were originally identified in families with autosomal recessive juvenile Parkinsonism (ARJP) [5]. It is the second most commonly affected PD gene and encodes a ubiquitin ligase associated with proteasomal degradation [55–57]. Since this gene is well conserved in Drosophila, several groups generated *parkin* null mutants in order to understand its biological role in flies. Although these mutants are viable, loss of Drosophila *parkin* function results in mitochondrial defects, degeneration of indirect flight muscles, hypersensitivity to oxidative and environmental stress, male sterility, reduced lifespan, partial lethality, and severe defects in both flight and climbing abilities [52, 58, 59]. It seems that oxidative stress, perhaps as a consequence of mitochondrial dysfunction, is a major determinant of those phenotypes [52, 60, 61]. Furthermore, *parkin* seems to be essential for the morphology, function, and integrity of several clusters of DA neurons in the Drosophila brain [59, 62]. Thus, fly *parkin* mutants recapitulate some key features of ARJP, suggesting that the mechanisms of DA neurodegeneration in mutant flies could resemble those underlying DA neuron loss in ARJP. It was proposed that loss of *parkin* function may lead to accumulation of one or several of its numerous substrates in the brain thereby resulting in ER stress, which in turn may lead to DA neuron death [28]. Regarding this, there are two studies in Drosophila which suggest that abnormal accumulation of Parkin substrates in Parkin-deficient DA neurons could be one of the causes of neurodegeneration. First, overexpression of human Parkin-associated endothelin-like receptor (PAEL-R), a Parkin substrate protein [63], in flies induces DA neuron loss in the DMC [64]. However, no Drosophila ortholog of this Parkin substrate has been described. We also demonstrated that targeted expression of Septin 4, the Drosophila ortholog of the human Parkin substrate CDCrel-1 [57], in DA neurons also causes age-dependent disruption of DA integrity in the DMC [65]. Since this neurotoxicity was dependent on *parkin* function and both proteins were able to interact in vitro, our results suggest that Septin4 could be a genuine substrate of Parkin in Drosophila [65]. This was the first study showing that accumulation of a Parkin substrate in flies could account for DA neurodegeneration in Drosophila *parkin* mutants [65].

It is interesting to mention that overexpression of mutant but not wild-type human *parkin* in flies also led to progressive degeneration of DA neurons from several clusters accompanied by a progressive motor impairment. These data suggested a possible dominant mechanism underlying the pathological phenotypes caused by mutant *parkin* in Drosophila, which could directly exert neurotoxicity in vivo [66, 67].

3.3. PINK1. Mutations in *PINK1* are also associated with recessive Parkinsonism. This gene encodes a putative serine/threonine kinase with a mitochondrial targeting sequence [8]. A recent study has demonstrated that the kinase domain faces to the cytosol, where its physiological substrates may reside [68]. The Drosophila *PINK1* gene encodes a protein that contains the same domains as its human counterpart, and fly *PINK1* models of PD were generated by transposon-mediated mutagenesis and RNAi [69–72]. Interestingly, *PINK* mutant flies shared marked phenotypic similarities with *parkin* mutants. They also exhibited male sterility, muscle degeneration, hypersensitivity to oxidative stress, mitochondrial defects, reduced lifespan, and DA neuronal degeneration accompanied by locomotor defects. Indeed, genetic analysis demonstrated that *PINK1* and *parkin* are functionally related. They showed that *parkin* overexpression rescued *PINK1* mutant phenotypes, whereas *PINK1* overexpression had no effect on *parkin* LOF phenotypes [69, 70]. These observations suggested that *PINK1* and *parkin* function in the same pathway, with *parkin* acting downstream of *PINK1*, and it seems that this pathway is conserved between flies and mammals [73]. Several studies have demonstrated that both fly genes regulate different aspects of mitochondrial physiology, thus explaining the mitochondrial morphological defects observed in Drosophila *PINK1* and *parkin* mutants. By means of genetic interactions, they illustrated a role of the PINK1/Parkin pathway in the regulation of the mitochondrial remodelling process in the direction of promoting mitochondrial fission and/or inhibiting fusion in Drosophila muscle and neuronal tissues [74–77]. However, these results also suggested that both genes are not core components of the mitochondrial dynamics machinery since LOF of key regulators of this process causes lethality and, as indicated above, *PINK1* and *parkin* mutants are viable.

Thus, it has been proposed that they probably regulate additional aspects of mitochondrial function that also impact mitochondrial morphology [76]. Interestingly, these results contrast with a human cell-based study which demonstrates that the PINK1/Parkin pathway promotes mitochondrial fusion in mammals [78]. One explanation for this discrepancy may be the existence of species-specific differences although the final conclusion is that in both systems there is a disrupted balance between mitochondrial fusion and fission [77]. Furthermore, it has been shown that PINK1 directly phosphorylates Parkin to control its translocation to the mitochondria [78]. Recent studies suggest that Parkin, together with PINK1, modulates mitochondrial trafficking, especially to the perinuclear region, a subcellular area associated with autophagy [79] and that PINK1 accumulation on mitochondria is both necessary and sufficient for Parkin recruitment to such organelles. These findings provide a biochemical explanation for the genetic epistasis found between *PINK1* and *parkin* in Drosophila and support a model in which PINK1 signals mitochondrial dysfunction to Parkin, and Parkin promotes their elimination [79, 80].

Genetic interaction experiments in flies also revealed putative additional components of the PINK1/Parkin pathway like Rhomboid-7 and Omi/HtrA2 [81, 82]. It seems that Rhomboid-7, a mitochondrial protease, could act as an upstream component of the pathway that may cleave the mitochondrial target motif of PINK1 thus allowing its activity not only in the mitochondria but also in the cytosol [81]. Besides, *Omi/HtrA2* was identified as a possible regulator of the PINK1/Parkin pathway, acting downstream of *PINK1* in Drosophila [82]. In contrast, another study showed that *Omi/HtrA2* does not play any role in the PINK1/Parkin pathway [83]. Although *Omi/HtrA2* sequence variations have been associated with an increased risk for PD [11, 84], its involvement in the disease is still controversial [12]. Additional work in Drosophila suggested that *PINK* deficiency also affects synaptic function in neurons, as the reserve pool of synaptic vesicles is not mobilized during rapid stimulation [85].

3.4. DJ-1. Mutations in the *DJ-1* gene are associated with rare familial recessive forms of PD [7]. *DJ-1* encodes a highly conserved protein belonging to the ThiJ/PfPI superfamily of molecular chaperones [86]. Although originally identified as an oncogenic factor [87], DJ-1 is a ubiquitous redox-responsive cytoprotective protein with diverse functions that, particularly in its oxidized form, has been recognized as a biomarker for cancer and neurodegenerative diseases [88]. Several cysteine residues in the DJ-1 protein can be oxidized with exposure to oxidative stress agents, being cysteine 106 critically required for DJ-1 to protect against oxidative damage both in vivo and in vitro [89, 90]. It has been shown that DJ-1 regulates redox signaling kinase pathways and acts as a transcriptional regulator of antioxidative gene batteries [91], but also acts as a redox-sensitive RNA-binding protein [92]. In contrast to mammalian species, two *DJ-1* orthologs do exist in Drosophila, *DJ-1α* and *DJ-1β*. While *DJ-1α* expression is restricted to the male germline, *DJ-1β* is ubiquitously expressed as its human counterpart [93, 94]. In order to explore the contribution of *DJ-1* in PD pathogenesis, we and others generated different Drosophila PD models by mutating these genes [50, 53, 93–95]. Those studies have revealed that flies mutant for *DJ-1α*, *DJ-1β*, or both are viable but exhibit enhanced sensitivity to toxins that induce oxidative stress such as H_2O_2, paraquat or rotenone, supporting that DJ-1 exerts a protective role against oxidative stress damage [50, 53, 93–95]. Consistent with this, we examined *DJ-1β* mutant flies for the extent of oxidative damage finding that *DJ-1β* loss of function results in cellular accumulation of reactive oxygen species (ROS) in adult brains, elevated levels of lipid peroxidation, and an increased catalase enzymatic activity [54]. It was also demonstrated that both the aging process and oxidation challenge promote overoxidation of DJ-1β at cysteine 104 (analogous to cysteine 106 in human DJ-1), a modification that could irreversibly inactivate the protein [90]. Consistent with this, aged flies showed further vulnerability to oxidative stress [90]. This suggests that the protective function of DJ-1 against oxidative stress could be progressively lost through aging, thus increasing the risk of DA neuron loss, since they are prone to oxidation. Despite this, only two studies have shown that targeted knockdown of *DJ-1α* via RNAi in flies resulted in age-dependent loss of DA neurons in the DMC [50, 53]. In addition, flies mutant for *DJ-1α* and *DJ-1β* showed reduced lifespan and locomotor defects [53, 95]. Although initial studies did not examine the *DJ-1* mutant flies for mitochondrial pathology that could account for these phenotypes, a recent analysis has demonstrated that *DJ-1* inactivation leads to mitochondrial dysfunction in an age-dependent manner not only in flies but also in mice [96]. Indeed, flies double mutant for *DJ-1α* and *DJ-1β* manifest additional phenotypes that reflect mitochondrial dysfunction such as reduced ATP levels and defects in spermatogenesis [96]. Interestingly, all these defects resemble those found in *parkin* and *PINK1* mutants (see Sections 3.2 and 3.3). Consistent with this, the study provides evidence that *DJ-1* interacts with the PINK1/Parkin pathway in Drosophila, and suggests that *DJ-1* acts downstream of, or in parallel to, *PINK1* for proper mitochondrial function [96]. Cell culture studies revealed that a pool of DJ-1 is localized to the mitochondria [89, 97]. Thus, all these results suggest that *DJ-1*, *parkin*, and *PINK1* may act in common biological processes that are critical for mitochondrial function and that *DJ-1* dysfunction may lead to PD pathology through distinct molecular mechanisms.

3.5. LRRK2. Mutations in *LRRK2* are likely the most common genetic cause of PD and are associated with a dominant form of the disease [9, 10]. It encodes a large and complex protein containing several independent domains, including a GTPase domain and a kinase domain able to exhibit a GTP-dependent phosphorylation activity [98]. The exact mechanism by which *LRRK2* mutations cause PD is still unclear. Most disease-associated mutations of LRRK2 have been shown to increase its kinase activity and thereby its toxicity, but there is significant variation among different mutations which can even reduce its kinase activity or exhibit a tendency to aggregate [99–101]. In order to understand the

mechanisms of *LRRK2*-induced pathology, several groups have used Drosophila to model *LRRK2*-linked Parkinsonism. Expression of either wild-type or mutant forms of human LRRK2 in flies has led to inconsistent results, especially regarding neurodegeneration [102–106]. While one group did not obtain any significant defect in the tissues analyzed, including muscles and DA neurons [102], other studies reported photoreceptor and/or DA neuron loss by LRRK2 overexpression as well as locomotor impairments [103–106]. Moreover, it was shown that human LRRK2 expression sensitized flies to environmental toxins such as rotenone [106]. Interestingly, LRRK2-overexpression phenotypes in fly eyes and DA neurons were modified in a complex fashion by a concomitant expression of *PINK1*, *DJ-1*, or *parkin*, suggesting a genetic interaction between these PD-relevant genes [106]. Regarding this, co-immunoprecipitation assays performed in cell culture already demonstrated that LRRK2 interacts with Parkin but not with α-Synuclein, DJ-1, or Tau in human cells [107]. Disparate results have also been obtained when ablating endogenous *LRRK2* expression in flies [102, 104, 108]. Several studies showed that flies lacking *LRRK2* function showed no changes in DA neuron numbers and patterns thus indicating that the gene is dispensable for the survival of DA neurons in this organism [104, 108]. However, one study reported that DA neurons in *LRRK2* LOF mutants show a severe reduction in tyrosine hydroxylase immunostaining and shrunken morphology, implicating their degeneration, and exhibit a severely impaired locomotive activity [102]. Different results have been also obtained when exposing those mutants to oxidative stress agents. While *LRRK2* mutants encoding a truncated form of the protein were selectively sensitive to hydrogen peroxide, but not to paraquat, rotenone and β-mercaptoethanol [108], *LRRK2* deficient (by transposon insertion or chromosome deletion), or *LRRK2* RNAi animals were shown to be significantly more resistant to hydrogen peroxide-induced stress [104]. Interestingly, this study also provided genetic and biochemical evidence that the Drosophila LRRK2 kinase modulates the maintenance of DA neurons by regulating protein synthesis, since it can phosphorylate initiation factor 4E-binding protein (4E-BP), a negative regulator of eukaryotic protein translation implicated in mediating the survival response to various physiological stresses [109–111]. Its phosphorylation relieves its inhibition of protein translation which could be detrimental when unregulated in times of stress. This would explain why flies expressing pathogenic forms of LRRK2 exhibit enhanced sensitivity to oxidative stress agents while flies lacking LRRK2 activity are resistant [104]. Consistent with this, it has been recently demonstrated that LRKK2 interacts with the microRNA pathway to regulate protein synthesis [112]. It is interesting to mention that a genetic interaction between 4E-BP (*Thor*) and *parkin/PINK1* has also been found, because its loss of function in *Drosophila* significantly reduces *parkin* and *PINK1* mutants viability while 4E-BP overexpression is sufficient to suppress the phenotypes described in these mutants [113]. Thus, these results support a general role of deregulated protein translation in PD. Besides, a recent study has shown that LRRK2 also phosphorylates the forkhead box transcription factor FoxO and enhances its transcriptional activity, not only in Drosophila but also in humans [114]. They also demonstrated that *hid* and *bim*, which encode two cell death molecules regulated by FoxO, are responsible for LRRK2-mediated cell death suggesting that they are key factors during the neurodegeneration in *LRRK2*-linked PD [114]. In summary, it seems that the higher kinase activity exhibited by LRRK2 mutations could cause DA neuron loss by affecting different cellular processes.

4. Using Drosophila Models to Study Molecular Mechanisms Underlying PD

The main goal of establishing animal models of human diseases is to provide new insights into their pathogenic mechanisms. To address this, Drosophila offers a wide variety of genetics tools. One of them is the possibility to perform genetic screens, which allow genome-wide analyses of genetic interactions based on the dominant modification of a given phenotype obtained by loss or gain of function of the gene of interest. Besides, a candidate gene approach can also be performed, in which only those genes that are suspected to be related to the PD-linked gene are assayed for modifications of the phenotype. Both strategies have allowed identifying components of multiple signaling pathways involved in PD pathogenesis. As seen in section 3, some PD-related phenotypes obtained in the fly models are not externally visible as is the case of DA neurons loss. Genetic interaction assays and genetic screens based on such phenotypes are often unaffordable and time consuming. Then, other phenotypes caused by mutations of the PD-related gene, which are easy to score and quantify, are used in the assays. Here, we report several examples of the identification of genes and signaling pathways involved in PD pathogenesis by means of genetic interaction assays performed in flies (see Table 1). Similar genetic experiments have been performed to determine functional relationships among some of the PD-related genes (see Section 3).

In order to identify the molecular mechanisms underlying the pathology associated with loss of function of fly *parkin* (see Section 3.2), a genetic screen for modifiers of the partial lethality phenotype of Drosophila *parkin* mutants was performed. This study identified an LOF allele of the *glutathione S-transferase S1* (*GstS1*) gene as the stronger enhancer of that phenotype [115]. Consistent with this, it was found that reducing *GstS1* activity was able to enhance DA neuron loss in *parkin* mutants while *GstS1* overexpression significantly suppressed that phenotype [62]. Since members of the GST family have been involved in detoxification of ROS [121], these data suggested a connection between *parkin* and oxidative stress response. This hypothesis was confirmed when analyzing the transcriptional profile of *parkin* mutant flies, which showed that an elevated percentage of deregulated genes in the mutants have functions related to oxidative stress response [115].

The importance of glutathione metabolism on DA neuron survival was also demonstrated in a posterior study based on a candidate gene approach. It showed that LOF mutants of genes involved in glutathione synthesis (*Eip55E* and the

Table 1: Signaling pathways and molecular processes involved in PD pathogenesis that have been identified by using Drosophila PD models.

Pathway/process	Drosophila model	Interacting genes/toxins	References
Oxidative stress	*parkin*	*GstS1*	[62, 115]
		Paraquat	[58]
	α-synuclein	*GstS1, Eip55E* and *Gclm*	[39]
		MsrA/Eip71CD	[38]
		Sod	[40]
	DJ-1α/β	Paraquat	[53, 90, 93–95]
		Rotenone	[93]
		H_2O_2	[50, 94]
	LRRK2/4E-BP	Paraquat, H_2O_2	[104]
	PINK1	*Sod*	[72]
		Rotenone	[69]
		Paraquat	[69, 72]
PI3K/Akt signaling	*DJ-1α/β*	*PTEN, Dp110*	[50]
Ras/ERK signaling	*DJ-1α/β*	*Ret, rl*	[116]
JNK signaling	*parkin*	*bsk, hep, puc*	[59]
DA metabolism	Paraquat	*ple, Pu, Catsup*	[27]
	parkin	*VMAT*	[66]
Mitochondrial structure and function	*PINK1*	*parkin*	[69, 70, 74–76, 78]
TOR signaling	*parkin/PINK1*	*4E-BP*	[82]
Removal of excess or toxic protein forms	*α-synuclein*	*Hsp70*	[117]
		ubiquitin	[51]
		dHDAC6	[118]
		SIRT2	[119]
		ctsd	[120]
	parkin	*PAEL-R*	[64]
		Sept4	[65]

Gcl-modifying subunit, Gclm) or glutathione conjugation pathways (*GstS1*) enhanced DA neuron loss of α-Synuclein-overexpressing flies while their overexpression suppressed that phenotype. Those genes were previously isolated in a genetic screen using a yeast model of α-synucleinopathy [37, 122]. The results obtained in this study indicated that α-Synuclein toxicity inversely correlates with the abundance of glutathione and GstS1 and suggest a role for Phase II detoxification pathway in PD pathogenesis [39]. Several studies have also dealt with the importance of α-Synuclein oligomers removal from the DA neuron cytoplasm to keep their integrity. The finding that progressive loss of DA neuron integrity produced by α-Synuclein overexpression is preventable in flies through directed expression of Hsp70 strongly suggested that eliminating toxic forms or excess of the protein could be central to prevent neuron damage [117]. Recently, coexpression of ubiquitin has been shown to rescue DA neuron degeneration and locomotor dysfunction in α-Synuclein-overexpressing flies. This neuroprotection is dependent on the formation of lysine 48 polyubiquitin linkage which is known to target protein degradation via the proteasome [51] and suggests that an increase of α-Synuclein targeting for degradation is able to reduce its toxicity. The involvement of histone deacetylase 6 (dHDAC6) in α-Synuclein toxicity was also analyzed [118], due to its role on sensing ubiquitinated aggregates and consequently activating chaperones expression, facilitating aggresome formation, and determining the fate of ubiquitinated proteins [123–125]. The authors found that knocking down the *dHDAC6* gene on α-Synuclein-overexpressing flies increased the amount of α-Synuclein oligomers while decreased the number of cytoplasmatic inclusions and DA neurons, indicating that dHDAC6 protects DA neuron integrity via promoting α-Synuclein inclusion formation [118]. These results support the role of LB as a successful defense against the concentration of toxic protein forms. Interestingly, inhibition of another protein of the histone deacetylase family, Sirtuin 2 (SIRT2), was also found to protect against α-Synuclein toxicity in *Drosophila* [119]. Finally, another study reported that deletion of the *ctsd* gene, which encodes the lysosomal protease Cathepsin D, promoted the retinal degeneration observed when in α-Synuclein overexpressing flies, suggesting that this protease may act as a facilitator of α-Synuclein-degrading activity [120].

DA neuron degeneration is one of the most distinguishing features of PD. For this reason, it seemed reasonable that genes involved in cell survival/death could have a role in PD pathogenesis. One study tackled this question by performing genetic interaction assays between *DJ-1α* and candidate genes or signaling pathways previously implicated in cell survival. This study led to identify genes in the PI3K/Akt signaling pathway as specific modifiers of the *DJ-1α*-associated cell death phenotype. Consistent with the genetic interaction results, they found that PI3K/Akt signaling regulates cellular ROS levels and that *DJ-1α* downregulation leads to PI3K/Akt signaling impairment. The same effect was observed in *parkin* mutants, thus suggesting a common molecular event between the two models [50]. These results are in contrasts with those obtained in a recent study that reported no interaction between *DJ-1α/β* and PI3K/Akt in the fly eye [116]. The authors described an interaction between *Ret*, a potent activator of both PI3K/Akt and Ras/ERK pathways, and *DJ-1α/β* in *Drosophila*. However, this interaction in the fly eye seems to be mediated by Ras/ERK [116]. The discrepancies could be due to the different systems used on each study, although further work would be necessary to uncover the real connection between *DJ-1α/β* and PI3K/Akt signaling. A relationship between *parkin* and other apoptosis signaling pathways has also been reported [59, 126]. These studies showed that *parkin* LOF mutants exhibit JNK pathway activation in DA neurons and that downregulation of this pathway is able to rescue the DA neuron loss phenotype observed in these mutants [59]. Genetic interactions between *parkin* and members of the JNK pathway also suggested that *parkin* is a negative regulator of this pathway and that this regulation is driven by a reduction in *basket* transcriptional levels [59, 126].

Several genetic studies in Drosophila have also shown that variations in genes regulating dopamine homeostasis, which are conserved in humans but not known to be associated with familial PD, can modify the neurodegeneration phenotype observed in the PD models and alter susceptibility to paraquat, a known environmental PD risk factor [27]. Although it has been extensively discussed, no agreement on the beneficial/toxic effect of this molecule on DA neuron survival and consequently on PD patients has been achieved. Some *in vitro* studies suggest that treatment with L-dopamine, the most common palliative pharmacological compound used in PD patients, could be toxic to DA neurons due to the activation of oxidative cascades produced by an increase in dopamine levels [127–129]. Moreover, an elevation of dopamine synthesis in response to a variety of stressors may expose DA neurons to high levels of oxidative stress [130–132]. In such a scenario, it has been shown that hyperactivated dopamine synthesis in Drosophila *cathecolamines up* (*catsup*) mutants, which might be expected to place the organism under high levels of oxidative stress, is instead able to provide protection against the effects of paraquat exposure. In contrast, compromised dopamine synthesis enhances susceptibility to paraquat-induced oxidative stress [27], thus indicating that sensitivity to paraquat might be modified by variations in genes that regulate dopamine synthesis and metabolism. Moreover, other study has shown that overexpression of the *Drosophila* vesicular monoamine transporter (VMAT), which regulates cytosolic DA homeostasis, partially rescues the degenerative phenotypes caused by overexpression of human *parkin* mutants while its knockdown exacerbates these phenotypes [66]. These result indicate that Parkin-induced neurotoxicity results from the interaction of mutant human *parkin* with cytoplasmic dopamine.

5. Using Drosophila PD Models to Identify Potentially Therapeutic Compounds

Both the genetic and toxin-induced Drosophila PD models represent a promising system for therapeutic compound identification. Indeed, during the last decade the effect of several compounds has been analyzed on behavioural, neurodegenerative or biochemical phenotypes of such models leading to the identification of potentially therapeutic compounds that could alleviate PD symptoms (see Table 2). Although candidate compounds have been always used in these studies, they open the possibility of performing high throughput compound screens which will be undoubtedly useful for finding new drugs that could alleviate PD symptoms.

The first published study about compound treatments in a Drosophila PD model reported the effects of drugs commonly used for treating PD on the locomotor phenotype of *α*-Synuclein expressing flies and showed that some of them were able to suppress that phenotype [133]. Subsequently, and given the ability of increased chaperone activity to counteract *α*-Synuclein toxicity [117], the effect of Geldanamycin (GA), an antibiotic able to interfere with Hsp90 activity and activate stress response, was assayed over *α*-Synuclein expressing flies [35, 134]. Notably, feeding these flies with GA protected DA neurons against *α*-Synuclein induced degeneration, and this protection was driven by an increase in Hsp70 levels [134]. Inhibitors of the histone deacetylase SIRT2 also showed a protective effect against *α*-Synuclein toxicity [119].

Other studies have been also performed in several Drosophila PD models to look for potentially therapeutic compounds directed to reduce oxidative stress damage. As explained previously, the study of *α*-Synuclein toxicity in flies led to the identification of Phase II detoxification pathway as a possible target for therapeutic treatment [39]. In fact, feeding *α*-Synuclein-expressing flies or *Drosophila parkin* mutants with pharmacological inducers of that pathway like sulforaphane or allyl disulfide suppresses the neuronal loss of both PD models [39]. These findings raise the possibility that these and perhaps other chemical inducers of Phase II detoxification pathway may represent potential preventive agents for PD. Besides, it has been shown that dietary supplementation with *S*-methyl-*L*-cysteine (SMLC) inhibits the locomotor and circadian rhythm defects caused by ectopic expression of human *α*-Synuclein in Drosophila [38]. SMLC participates in the catalytic antioxidant mechanism involving Methionine sulfoxide reductase A (MSRA), one of the enzymes that catalyze the oxidation of the amino acid methionine to methionine sulfoxide, a reversible reaction

Table 2: Potentially therapeutic compounds able to modify different phenotypes in the Drosophila PD models.

Pathway/process	Compound treatment*	Drosophila model	Modified phenotype/s	References
Oxidative stress	Sulforaphane and allyl disulfide	*parkin*	DA neuron number	[39]
		α-synuclein	DA neuron number	[39]
	S-methyl-*L*-cysteine	*α-synuclein*	Locomotor activity	[38]
	Polyphenols	*α-synuclein*	Lifespan, locomotor activity	[135]
		Paraquat and iron	Locomotor activity	[136]
	α-tocopherol	*DJ-1β*	Lifespan	[54]
		PINK1	Ommatidial degeneration	[72]
	SOD	*PINK1*	Ommatidial degeneration	[72]
	Melatonin	*DJ-1β*	Lifespan	[54]
		Paraquat	Locomotor activity	[27]
		Rotenone	Locomotor activity, DA neuron number	[27]
	Bacopa monieri leaf extract	Paraquat	Oxidative markers levels	[137]
Oxidative stress/inflammatory process	Minocycline	*DJ-1α*	DA neuron number, dopamine levels	[138]
	Celastrol	*DJ-1α*	DA neuron number, dopamine levels, locomotor activity, and survival rate under oxidative stress conditions	[138]
TOR signaling	Rapamycin	*parkin/PINK1*	Thoracic indentations, locomotor activity, DA neuron number, and muscle integrity	[82]
Removal of excess or toxic protein forms	Geldanamycin	*α-synuclein*	DA neuron number	[35, 134]
Zinc homeostasis	Zinc chloride	*parkin*	Lifespan, locomotor activity, and percentage of adulthood survivors	[139]

* All treatments were administered as dietary complement.

that has been postulated to act protecting cells from oxidative damage. Furthermore, grape extract supplementation has been shown to recover locomotor ability and lifespan in *α*-Synuclein-expressing flies. It is known that grape extracts contain several polyphenols, compounds with antioxidant properties [135]. Other Drosophila PD models in which treatments with antioxidant compounds have been shown to be beneficial are those involving the *DJ-1α* and *DJ-1β* genes [54, 138]. Compounds with antioxidant and anti-inflammatory properties such as celastrol and minocycline conferred potent DA neuroprotection in RNAi *DJ-1α* mutants [138]. We have also recently demonstrated that chronic treatments with antioxidant compounds are able to modify the lifespan phenotype of *DJ-1β* mutant flies, thus suggesting that oxidative stress plays a causal role in such phenotype [54].

It is known that rapamycin is a small molecule inhibitor of TOR signaling that has been shown to lead to 4E-BP hypophosphorylation in vitro and in vivo [140, 141]. Notably rapamycin administration was able to suppress all pathologic phenotypes in *park* and *PINK1* mutants. Moreover, this suppression was found to be 4E-BP-dependent, since the administration of rapamycin to *parkin* and *Thor* or *PINK1* and *Thor* double mutants was completely unable to suppress these phenotypes [113]. Since 4E-BP activity can be manipulated by small molecule inhibitors such as rapamycin, this pathway represents a viable therapeutic target for PD treatment. Moreover, it has been recently suggested that *parkin* mutants, apart from the described phenotypes, also present altered zinc homeostasis. This is supported by the fact that dietary zinc supplementation in the form of zinc chloride increased lifespan as well as the percentage of *parkin* mutant flies reaching adulthood while this supplemented diet was deleterious to control flies [139].

Since most PD cases are sporadic and could be associated to different environmental agents, it is also essential the use of toxin-induced Drosophila PD models to assay the beneficial effects of candidate compounds. Polyphenol administration was also found to exert a beneficial effect on flies exposed to paraquat and iron, protecting, rescuing, and restoring the impaired locomotor activity caused by exposure to those agents [136]. Other antioxidant compounds such as melatonin have also been found to rescue locomotor deficits and DA neurodegeneration in flies exposed to rotenone [26]. Similarly, it has been recently reported that oxidative perturbations, measured by different oxidative markers,

induced by paraquat exposure in *Drosophila* are mitigated by treatment with leaf extracts of *Bacopa monieri*, an Indian herb with attributed neuroprotective functions [137].

6. Conclusions

As reported in this review, Drosophila has emerged as a very valuable model organism to study PD. Although it is impossible to fully recapitulate the key neuropathologic and clinical features of human PD in a single model organism, many of the existing PD models in Drosophila exhibit key features of the disease and have provided insights into PD pathogenesis. Either toxin-induced PD models or models based on mutations in genes that are linked to familial PD have provided the proper context by which conserved signaling pathways and molecular processes relevant to the disease are discovered and compounds able to suppress PD-related phenotypes in flies are discovered as well. Indeed, Drosophila PD models represent a promising system for the identification of new genes that could be involved in PD susceptibility/development as well as of therapeutic compound that could be relevant to alleviate PD symptoms in humans.

References

[1] J. Jankovic, "Parkinson's disease: clinical features and diagnosis," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 79, no. 4, pp. 368–376, 2008.

[2] A. J. Lees, J. Hardy, and T. Revesz, "Parkinson's disease," *The Lancet*, vol. 373, no. 9680, pp. 2055–2066, 2009.

[3] J. Bové, D. Prou, C. Perier, and S. Przedborski, "Toxin-induced models of Parkinson's disease," *NeuroRx*, vol. 2, no. 3, pp. 484–494, 2005.

[4] M. H. Polymeropoulos, C. Lavedan, E. Leroy et al., "Mutation in the α-synuclein gene identified in families with Parkinson's disease," *Science*, vol. 276, no. 5321, pp. 2045–2047, 1997.

[5] T. Kitada, S. Asakawa, N. Hattori et al., "Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism," *Nature*, vol. 392, no. 6676, pp. 605–608, 1998.

[6] E. Leroy, R. Boyer, G. Auburger et al., "The ubiquitin pathway in Parkinson's disease," *Nature*, vol. 395, no. 6701, pp. 451–452, 1998.

[7] V. Bonifati, P. Rizzu, M. J. Van Baren et al., "Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism," *Science*, vol. 299, no. 5604, pp. 256–259, 2003.

[8] E. M. Valente, P. M. Abou-Sleiman, V. Caputo et al., "Hereditary early-onset Parkinson's disease caused by mutations in PINK1," *Science*, vol. 304, no. 5674, pp. 1158–1160, 2004.

[9] C. Paisán-Ruíz, S. Jain, E. W. Evans et al., "Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease," *Neuron*, vol. 44, no. 4, pp. 595–600, 2004.

[10] A. Zimprich, S. Biskup, P. Leitner et al., "Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology," *Neuron*, vol. 44, no. 4, pp. 601–607, 2004.

[11] K. M. Strauss, L. M. Martins, H. Plun-Favreau et al., "Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease," *Human Molecular Genetics*, vol. 14, no. 15, pp. 2099–2111, 2005.

[12] J. Simón-Sánchez and A. B. Singleton, "Sequencing analysis of OMI/HTRA2 shows previously reported pathogenic mutations in neurologically normal controls," *Human Molecular Genetics*, vol. 17, no. 13, pp. 1988–1993, 2008.

[13] A. Ramirez, A. Heimbach, J. Gründemann et al., "Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase," *Nature Genetics*, vol. 38, no. 10, pp. 1184–1191, 2006.

[14] L. N. Clark, B. M. Ross et al., "Mutations in the glucocerebrosidase gene are associated with early-onset Parkinson disease," *Neurology*, vol. 69, no. 12, pp. 1270–1277, 2007.

[15] K. L. Lim and C. H. Ng, "Genetic models of Parkinson disease," *Biochimica et Biophysica Acta*, vol. 1792, no. 7, pp. 604–615, 2009.

[16] J. B. Schulz, "Mechanisms of neurodegeneration in idiopathic Parkinson's disease," *Parkinsonism and Related Disorders*, vol. 13, no. 3, pp. S306–S308, 2007.

[17] T. M. Dawson, H. S. Ko, and V. L. Dawson, "Genetic animal models of Parkinson's disease," *Neuron*, vol. 66, no. 5, pp. 646–661, 2010.

[18] L. T. Reiter, L. Potocki, S. Chien, M. Gribskov, and E. Bier, "A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster," *Genome Research*, vol. 11, no. 6, pp. 1114–1125, 2001.

[19] B. Lu and H. Vogel, "Drosophila models of neurodegenerative diseases," *Annual Review of Pathology*, vol. 4, pp. 315–342, 2009.

[20] S. S. Ambegaokar, B. Roy, and G. R. Jackson, "Neurodegenerative models in Drosophila: polyglutamine disorders, Parkinson disease, and amyotrophic lateral sclerosis," *Neurobiology of Disease*, vol. 40, no. 1, pp. 29–39, 2010.

[21] F. Hirth, "Drosophila melanogaster in the study of human neurodegeneration," *CNS and Neurological Disorders*, vol. 9, no. 4, pp. 504–523, 2010.

[22] A. J. Whitworth, P. D. Wes, and L. J. Pallanck, "Drosophila models pioneer a new approach to drug discovery for Parkinson's disease," *Drug Discovery Today*, vol. 11, no. 3-4, pp. 119–126, 2006.

[23] J. A. Botella, F. Bayersdorfer, F. Gmeiner, and S. Schneuwly, "Modelling Parkinson's Disease in Drosophila," *Neuromolecular Medicine*, vol. 11, no. 4, pp. 268–280, 2009.

[24] R. Betarbet, T. B. Sherer, D. A. Di Monte, and J. T. Greenamyre, "Mechanistic approaches to Parkinson's disease pathogenesis," *Brain Pathology*, vol. 12, no. 4, pp. 499–510, 2002.

[25] R. K. Dagda, J. Zhu, and C. T. Chu, "Mitochondrial kinases in Parkinson's disease: converging insights from neurotoxin and genetic models," *Mitochondrion*, vol. 9, no. 5, pp. 289–298, 2009.

[26] H. Coulom and S. Birman, "Chronic exposure to rotenone models sporadic Parkinson's disease in Drosophila melanogaster," *Journal of Neuroscience*, vol. 24, no. 48, pp. 10993–10998, 2004.

[27] A. Chaudhuri, K. Bowling, C. Funderburk et al., "Interaction of genetic and environmental factors in a Drosophila parkinsonism model," *Journal of Neuroscience*, vol. 27, no. 10, pp. 2457–2467, 2007.

[28] J. Park, Y. Kim, and J. Chung, "Mitochondrial dysfunction and Parkinson's disease genes: insights from Drosophila," *DMM Disease Models and Mechanisms*, vol. 2, no. 7-8, pp. 336–340, 2009.

[29] A. H. Brand and N. Perrimon, "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes," *Development*, vol. 118, no. 2, pp. 401–415, 1993.

[30] R. Krüger, W. Kuhn, T. Müller et al., "Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease," *Nature Genetics*, vol. 18, no. 2, pp. 106–108, 1998.

[31] J. J. Zarranz, J. Alegre, J. C. Gómez-Esteban et al., "The new mutation, E46K, of α-synuclein causes Parkinson and Lewy body dementia," *Annals of Neurology*, vol. 55, no. 2, pp. 164–173, 2004.

[32] A. B. Singleton, M. Farrer, J. Johnson et al., "α-synuclein locus triplication causes Parkinson's disease," *Science*, vol. 302, no. 5646, p. 841, 2003.

[33] M. G. Spillantini, M. L. Schmidt, V. M. Y. Lee, J. Q. Trojanowski, R. Jakes, and M. Goedert, "α-synuclein in Lewy bodies," *Nature*, vol. 388, no. 6645, pp. 839–840, 1997.

[34] M. B. Feany and W. W. Bender, "A Drosophila model of Parkinson's disease," *Nature*, vol. 404, no. 6776, pp. 394–398, 2000.

[35] P. K. Auluck, M. C. Meulener, and N. M. Bonini, "Mechanisms of suppression of α-synuclein neurotoxicity by geldanamycin in Drosophila," *Journal of Biological Chemistry*, vol. 280, no. 4, pp. 2873–2878, 2005.

[36] Y. Pesah, H. Burgess, B. Middlebrooks et al., "Whole-mount analysis reveals normal numbers of dopaminergic neurons following misexpression of α-synuclein in Drosophila," *Genesis*, vol. 41, no. 4, pp. 154–159, 2005.

[37] A. A. Cooper, A. D. Gitler, A. Cashikar et al., "α-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models," *Science*, vol. 313, no. 5785, pp. 324–328, 2006.

[38] R. Wassef, R. Haenold, A. Hansel, N. Brot, S. H. Heinemann, and T. Hoshi, "Methionine sulfoxide reductase A and a dietary supplement S-methyl-L-cysteine prevent Parkinson's-like symptoms," *Journal of Neuroscience*, vol. 27, no. 47, pp. 12808–12816, 2007.

[39] K. Trinh, K. Moore, P. D. Wes et al., "Induction of the phase II detoxification pathway suppresses neuron loss in Drosophila models of Parkinson's disease," *Journal of Neuroscience*, vol. 28, no. 2, pp. 465–472, 2008.

[40] J. A. Botella, F. Bayersdorfer, and S. Schneuwly, "Superoxide dismutase overexpression protects dopaminergic neurons in a Drosophila model of Parkinson's disease," *Neurobiology of Disease*, vol. 30, no. 1, pp. 65–73, 2008.

[41] Z. Xun, R. A. Sowell, T. C. Kaufman, and D. E. Clemmer, "Protein expression in a Drosophila model of Parkinson's disease," *Journal of Proteome Research*, vol. 6, no. 1, pp. 348–357, 2007.

[42] Z. Xun, R. A. Sowell, T. C. Kaufman, and D. E. Clemmer, "Lifetime proteomic profiling of an A30P α-synuclein Drosophila model of Parkinson's disease," *Journal of Proteome Research*, vol. 6, no. 9, pp. 3729–3738, 2007.

[43] Z. Xun, R. A. Sowell, T. C. Kaufman, and D. E. Clemmer, "Quantitative proteomics of a presymptomatic A53T α-synuclein Drosophila model of Parkinson disease," *Molecular and Cellular Proteomics*, vol. 7, no. 7, pp. 1191–1203, 2008.

[44] Z. Xun, T. C. Kaufman, and D. E. Clemmer, "Proteome response to the panneural expression of human wild-type α-synuclein: a drosophila model of Parkinson's disease," *Journal of Proteome Research*, vol. 7, no. 9, pp. 3911–3921, 2008.

[45] M. Periquet, T. Fulga, L. Myllykangas, M. G. Schlossmacher, and M. B. Feany, "Aggregated α-synuclein mediates dopaminergic neurotoxicity in vivo," *Journal of Neuroscience*, vol. 27, no. 12, pp. 3338–3346, 2007.

[46] H. Fujiwara, M. Hasegawa, N. Dohmae et al., "α-synuclein is phosphorylated in synucleinopathy lesions," *Nature Cell Biology*, vol. 4, no. 2, pp. 160–164, 2002.

[47] L. Chen and M. B. Feany, "α-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease," *Nature Neuroscience*, vol. 8, no. 5, pp. 657–663, 2005.

[48] L. Chen, M. Periquet, X. Wang et al., "Tyrosine and serine phosphorylation of α-synuclein have opposing effects on neurotoxicity and soluble oligomer formation," *Journal of Clinical Investigation*, vol. 119, no. 11, pp. 3257–3265, 2009.

[49] N. Cavallarin, M. Vicario, and A. Negro, "The role of phosphorylation in synucleinopathies: focus on Parkinson's disease," *CNS and Neurological Disorders*, vol. 9, no. 4, pp. 471–481, 2010.

[50] Y. Yang, S. Gehrke, M. E. Haque et al., "Inactivation of Drosophila DJ-1 leads to impairments of oxidative stress response and phosphatidylinositol 3-kinase/Akt signaling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 38, pp. 13670–13675, 2005.

[51] F. K. M. Lee, A. K.Y. Wong, Y. W. Lee, O. W. Wan, H. Y. Edwin Chan, and K. K.K. Chung, "The role of ubiquitin linkages on α-synuclein induced-toxicity in a Drosophila model of Parkinson's disease," *Journal of Neurochemistry*, vol. 110, no. 1, pp. 208–219, 2009.

[52] J. C. Greene, A. J. Whitworth, I. Kuo, L. A. Andrews, M. B. Feany, and L. J. Pallanck, "Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 7, pp. 4078–4083, 2003.

[53] E. Lavara-Culebras and N. Paricio, "Drosophila DJ-1 mutants are sensitive to oxidative stress and show reduced lifespan and motor deficits," *Gene*, vol. 400, no. 1-2, pp. 158–165, 2007.

[54] E. Lavara-Culebras, V. Muñoz-Soriano, R. Gómez-Pastor, E. Matallana, and N. Paricio, "Effects of pharmacological agents on the lifespan phenotype of Drosophila DJ-1β mutants," *Gene*, vol. 462, no. 1-2, pp. 26–33, 2010.

[55] Y. Imai, M. Soda, and R. Takahashi, "Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity," *Journal of Biological Chemistry*, vol. 275, no. 46, pp. 35661–35664, 2000.

[56] H. Shimura, N. Hattori, S. I. Kubo et al., "Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase," *Nature Genetics*, vol. 25, no. 3, pp. 302–305, 2000.

[57] Y. Zhang, J. Gao, K. K. K. Chung, H. Huang, V. L. Dawson, and T. M. Dawson, "Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 24, pp. 13354–13359, 2000.

[58] Y. Pesah, T. Pham, H. Burgess et al., "Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress," *Development*, vol. 131, no. 9, pp. 2183–2194, 2004.

[59] G. H. Cha, S. Kim, J. Park et al., "Parkin negatively regulates JNK pathway in the dopaminergic neurons of Drosophila," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 29, pp. 10345–10350, 2005.

[60] M. G. Riparbelli and G. Callaini, "The Drosophila parkin homologue is required for normal mitochondrial dynamics during spermiogenesis," *Developmental Biology*, vol. 303, no. 1, pp. 108–120, 2007.

[61] N. Saini, S. Oelhafen, H. Hua, O. Georgiev, W. Schaffner, and H. Büeler, "Extended lifespan of Drosophila parkin mutants through sequestration of redox-active metals and

enhancement of anti-oxidative pathways," *Neurobiology of Disease*, vol. 40, no. 1, pp. 82–92, 2010.

[62] A. J. Whitworth, D. A. Theodore, J. C. Greene, H. Beneš, P. D. Wes, and L. J. Pallanck, "Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a Drosophila model of Parkinson's disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 22, pp. 8024–8029, 2005.

[63] Y. Imai, M. Soda, H. Inoue, N. Hattori, Y. Mizuno, and R. Takahashi, "An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin," *Cell*, vol. 105, no. 7, pp. 891–902, 2001.

[64] Y. Yang, I. Nishimura, Y. Imai, R. Takahashi, and B. Lu, "Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in Drosophila," *Neuron*, vol. 37, no. 6, pp. 911–924, 2003.

[65] V. Muñoz-Soriano and N. Paricio, "Overexpression of Septin 4, the Drosophila homologue of human CDCrel-1, is toxic for dopaminergic neurons," *European Journal of Neuroscience*, vol. 26, no. 11, pp. 3150–3158, 2007.

[66] T. K. Sang, H. Y. Chang, G. M. Lawless et al., "A Drosophila model of mutant human parkin-induced toxicity demonstrates selective loss of dopaminergic neurons and dependence on cellular dopamine," *Journal of Neuroscience*, vol. 27, no. 5, pp. 981–992, 2007.

[67] C. Wang, R. Lu, X. Ouyang et al., "Drosophila overexpressing parkin R275W mutant exhibits dopaminergic neuron degeneration and mitochondrial abnormalities," *Journal of Neuroscience*, vol. 27, no. 32, pp. 8563–8570, 2007.

[68] C. Zhou, Y. Huang, Y. Shao et al., "The kinase domain of mitochondrial PINK1 faces the cytoplasm," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 33, pp. 12022–12027, 2008.

[69] I. E. Clark, M. W. Dodson, C. Jiang et al., "Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin," *Nature*, vol. 441, no. 7097, pp. 1162–1166, 2006.

[70] J. Park, S. B. Lee, S. Lee et al., "Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin," *Nature*, vol. 441, no. 7097, pp. 1157–1161, 2006.

[71] Y. Yang, S. Gehrke, Y. Imai et al., "Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 28, pp. 10793–10798, 2006.

[72] D. Wang, L. Qian, H. Xiong et al., "Antioxidants protect PINK1-dependent dopaminergic neurons in Drosophila," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 36, pp. 13520–13525, 2006.

[73] N. Exner, B. Treske, D. Paquet et al., "Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin," *Journal of Neuroscience*, vol. 27, no. 45, pp. 12413–12418, 2007.

[74] A. C. Poole, R. E. Thomas, L. A. Andrews, H. M. McBride, A. J. Whitworth, and L. J. Pallanck, "The PINK1/Parkin pathway regulates mitochondrial morphology," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 5, pp. 1638–1643, 2008.

[75] Y. Yang, Y. Ouyang, L. Yang et al., "Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 19, pp. 7070–7075, 2008.

[76] H. Deng, M. W. Dodson, H. Huang, and M. Guo, "The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in Drosophila," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 38, pp. 14503–14508, 2008.

[77] J. Park, G. Lee, and J. Chung, "The PINK1-Parkin pathway is involved in the regulation of mitochondrial remodeling process," *Biochemical and Biophysical Research Communications*, vol. 378, no. 3, pp. 518–523, 2009.

[78] Y. Kim, J. Park, S. Kim et al., "PINK1 controls mitochondrial localization of Parkin through direct phosphorylation," *Biochemical and Biophysical Research Communications*, vol. 377, no. 3, pp. 975–980, 2008.

[79] C. Vives-Bauza, C. Zhou, Y. Huang et al., "PINK1-dependent recruitment of Parkin to mitochondria in mitophagy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 1, pp. 378–383, 2010.

[80] D. P. Narendra, S. M. Jin, A. Tanaka et al., "PINK1 is selectively stabilized on impaired mitochondria to activate Parkin," *PLoS Biology*, vol. 8, no. 1, article e1000298, 2010.

[81] A. J. Whitworth, J. R. Lee, V. M. W. Ho, R. Flick, R. Chowdhury, and G. A. McQuibban, "Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin," *DMM Disease Models and Mechanisms*, vol. 1, no. 2-3, pp. 168–174, 2008.

[82] L. S. Tain, R. B. Chowdhury, R. N. Tao et al., "Drosophila HtrA2 is dispensable for apoptosis but acts downstream of PINK1 independently from Parkin," *Cell Death and Differentiation*, vol. 16, no. 8, pp. 1118–1125, 2009.

[83] J. Yun, J. H. Cao, M. W. Dodson et al., "Loss-of-function analysis suggests that Omi/HtrA2 is not an essential component of the pink1/parkin pathway in vivo," *Journal of Neuroscience*, vol. 28, no. 53, pp. 14500–14510, 2008.

[84] V. Bogaerts, K. Nuytemans, J. Reumers et al., "Genetic variability in the mitochondrial serine protease HTRA2 contributes to risk for Parkinson disease," *Human Mutation*, vol. 29, no. 6, pp. 832–840, 2008.

[85] V. A. Morais, P. Verstreken, A. Roethig et al., "Parkinson's disease mutations in PINK1 result in decreased Complex I activity and deficient synaptic function," *EMBO Molecular Medicine*, vol. 1, no. 2, pp. 99–111, 2009.

[86] J. I. Lucas and I. Marín, "A new evolutionary paradigm for the Parkinson disease gene DJ-1," *Molecular Biology and Evolution*, vol. 24, no. 2, pp. 551–561, 2007.

[87] D. Nagakubo, T. Taira, H. Kitaura et al., "DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras," *Biochemical and Biophysical Research Communications*, vol. 231, no. 2, pp. 509–513, 1997.

[88] P. J. Kahle, J. Waak, and T. Gasser, "DJ-1 and prevention of oxidative stress in Parkinson's disease and other age-related disorders," *Free Radical Biology and Medicine*, vol. 47, no. 10, pp. 1354–1361, 2009.

[89] R. M. Canet-Avilés, M. A. Wilson, D. W. Miller et al., "The Parkinson's disease DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 24, pp. 9103–9108, 2004.

[90] M. C. Meulener, K. Xu, L. Thomson, H. Ischiropoulos, and N. M. Bonini, "Mutational analysis of DJ-1 in Drosophila implicates functional inactivation by oxidative damage and aging," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 33, pp. 12517–12522, 2006.

[91] N. Zhong and J. Xu, "Synergistic activation of the human MnSOD promoter by DJ-1 and PGC-1α: regulation by SUMOylation and oxidation," *Human Molecular Genetics*, vol. 17, no. 21, pp. 3357–3367, 2008.

[92] M. P. Van Der Brug, J. Blackinton, J. Chandran et al., "RNA binding activity of the recessive parkinsonism protein DJ-1 supports involvement in multiple cellular pathways," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 29, pp. 10244–10249, 2008.

[93] M. Meulener, A. J. Whitworth, C. E. Armstrong-Gold et al., "Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease," *Current Biology*, vol. 15, no. 17, pp. 1572–1577, 2005.

[94] F. M. Menzies, S. C. Yenisetti, and K. T. Min, "Roles of Drosophila DJ-1 in survival of dopaminergic neurons and oxidative stress," *Current Biology*, vol. 15, no. 17, pp. 1578–1582, 2005.

[95] J. Park, Y. K. Sung, G. H. Cha, B. L. Sung, S. Kim, and J. Chung, "Drosophila DJ-1 mutants show oxidative stress-sensitive locomotive dysfunction," *Gene*, vol. 361, no. 1-2, pp. 133–139, 2005.

[96] L. Y. Hao, B. I. Giasson, and N. M. Bonini, "DJ-1 is critical for mitochondrial function and rescues PINK1 loss of function," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 21, pp. 9747–9752, 2010.

[97] L. Zhang, M. Shimoji, B. Thomas et al., "Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis," *Human Molecular Genetics*, vol. 14, no. 14, pp. 2063–2073, 2005.

[98] A. B. West, D. J. Moore, S. Biskup et al., "Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 46, pp. 16842–16847, 2005.

[99] W. W. Smith, Z. Pei, H. Jiang, V. L. Dawson, T. M. Dawson, and C. A. Ross, "Kinase activity of mutant LRRK2 mediates neuronal toxicity," *Nature Neuroscience*, vol. 9, no. 10, pp. 1231–1233, 2006.

[100] E. Greggio, S. Jain, A. Kingsbury et al., "Kinase activity is required for the toxic effects of mutant LRRK2/dardarin," *Neurobiology of Disease*, vol. 23, no. 2, pp. 329–341, 2006.

[101] A. B. West, D. J. Moore, C. Choi et al., "Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity," *Human Molecular Genetics*, vol. 16, no. 2, pp. 223–232, 2007.

[102] S. B. Lee, W. Kim, S. Lee, and J. Chung, "Loss of LRRK2/PARK8 induces degeneration of dopaminergic neurons in Drosophila," *Biochemical and Biophysical Research Communications*, vol. 358, no. 2, pp. 534–539, 2007.

[103] Z. Liu, X. Wang, Y. Yu et al., "A Drosophila model for LRRK2-linked parkinsonism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 7, pp. 2693–2698, 2008.

[104] Y. Imai, S. Gehrke, H. Q. Wang et al., "Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in Drosophila," *EMBO Journal*, vol. 27, no. 18, pp. 2432–2443, 2008.

[105] C. H. Ng, S. Z. S. Mok, C. Koh et al., "Parkin protects against LRRK2 G2019S mutant-induced dopaminergic neurodegeneration in Drosophila," *Journal of Neuroscience*, vol. 29, no. 36, pp. 11257–11262, 2009.

[106] K. Venderova, G. Kabbach, E. Abdel-Messih et al., "Leucine-rich repeat kinase 2 interacts with Parkin, DJ-1 and PINK-1 in a Drosophila melanogaster model of Parkinson's disease," *Human Molecular Genetics*, vol. 18, no. 22, pp. 4390–4404, 2009.

[107] W. W. Smith, Z. Pei, H. Jiang et al., "Leucine-rich repeat kinase 2 (LRRK2) interacts with parkin, and mutant LRRK2 induces neuronal degeneration," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 51, pp. 18676–18681, 2005.

[108] D. Wang, B. Tang, G. Zhao et al., "Dispensable role of Drosophila ortholog of LRRK2 kinase activity in survival of dopaminergic neurons," *Molecular Neurodegeneration*, vol. 3, no. 1, article 3, 2008.

[109] M. J. Clemens, "Translational regulation in cell stress and apoptosis. Roles of the eIF4E binding proteins," *Journal of Cellular and Molecular Medicine*, vol. 5, no. 3, pp. 221–239, 2001.

[110] M. Holcik and N. Sonenberg, "Translational control in stress and apoptosis," *Nature Reviews Molecular Cell Biology*, vol. 6, no. 4, pp. 318–327, 2005.

[111] J. D. Richter and N. Sonenberg, "Regulation of cap-dependent translation by eIF4E inhibitory proteins," *Nature*, vol. 433, no. 7025, pp. 477–480, 2005.

[112] S. Gehrke, Y. Imai, N. Sokol, and B. Lu, "Pathogenic LRRK2 negatively regulates microRNA-mediated translational repression," *Nature*, vol. 466, no. 7306, pp. 637–641, 2010.

[113] L. S. Tain, H. Mortiboys, R. N. Tao, E. Ziviani, O. Bandmann, and A. J. Whitworth, "Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss," *Nature Neuroscience*, vol. 12, no. 9, pp. 1129–1135, 2009.

[114] T. Kanao, K. Venderova, D. S. Park, T. Unterman, B. Lu, and Y. Imai, "Activation of FoxO by LRRK2 induces expression of proapoptotic proteins and alters survival of postmitotic dopaminergic neuron in Drosophila," *Human Molecular Genetics*, vol. 19, no. 19, pp. 3747–3758, 2010.

[115] J. C. Greene, A. J. Whitworth, L. A. Andrews, T. J. Parker, and L. J. Pallanck, "Genetic and genomic studies of Drosophila parkin mutants implicate oxidative stress and innate immune responses in pathogenesis," *Human Molecular Genetics*, vol. 14, no. 6, pp. 799–811, 2005.

[116] L. Aron, P. Klein, T. T. Pham, E. R. Kramer, W. Wurst, and R. Klein, "Pro-survival role for Parkinson's associated gene DJ-1 revealed in trophically impaired dopaminergic neurons," *PLoS Biology*, vol. 8, no. 4, article e1000349, 2010.

[117] P. K. Auluck, H. Y. E. Chan, J. Q. Trojanowski, V. M.-Y. Lee, and N. M. Bonini, "Chaperone suppression of α-synuclein toxicity in a Drosophila model for Parkinson's disease," *Science*, vol. 295, no. 5556, pp. 865–868, 2002.

[118] G. Du, X. Liu, X. Chen et al., "Drosophila histone deacetylase 6 protects dopaminergic neurons against α-synuclein toxicity by promoting inclusion formation," *Molecular Biology of the Cell*, vol. 21, no. 13, pp. 2128–2137, 2010.

[119] T. F. Outeiro, E. Kontopoulos, S. M. Altmann et al., "Sirtuin 2 inhibitors rescue α-synuclein-mediated toxicity in models of Parkinson's disease," *Science*, vol. 317, no. 5837, pp. 516–519, 2007.

[120] V. Cullen, M. Lindfors, J. Ng et al., "Cathepsin D expression level affects alpha-synuclein processing, aggregation, and toxicity in vivo," *Molecular Brain*, vol. 2, no. 1, article 5, 2009.

[121] J. D. Hayes, J. U. Flanagan, and I. R. Jowsey, "Glutathione transferases," *Annual Review of Pharmacology and Toxicology*, vol. 45, pp. 51–88, 2005.

[122] S. Willingham, T. F. Outeiro, M. J. DeVit, S. L. Lindquist, and P. J. Muchowski, "Yeast genes that enhance the toxicity of a mutant huntingtin fragment or α-synuclein," *Science*, vol. 302, no. 5651, pp. 1769–1772, 2003.

[123] C. Boyault, Y. Zhang, S. Fritah et al., "HDAC6 controls major cell response pathways to cytotoxic accumulation of protein aggregates," *Genes and Development*, vol. 21, no. 17, pp. 2172–2181, 2007.

[124] Y. Kawaguchi, J. J. Kovacs, A. McLaurin, J. M. Vance, A. Ito, and T. P. Yao, "The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress," *Cell*, vol. 115, no. 6, pp. 727–738, 2003.

[125] C. Boyault, B. Gilquin, Y. Zhang et al., "HDAC6-p97/VCP controlled polyubiquitin chain turnover," *EMBO Journal*, vol. 25, no. 14, pp. 3357–3366, 2006.

[126] S. Hwang, D. Kim, G. Choi et al., "Parkin Suppresses c-Jun N-terminal kinase-induced cell death via transriptional regulation in Drosophila," *Molecules and Cells*, vol. 29, no. 6, pp. 575–580, 2010.

[127] A. H. Stokes, T. G. Hastings, and K. E. Vrana, "Cytotoxic and genotoxic potential of dopamine," *Journal of Neuroscience Research*, vol. 55, no. 6, pp. 659–665, 1999.

[128] B. Pardo, M. A. Mena, M. J. Casarejos, C. L. Paino, and J. G. De Yebenes, "Toxic effects of L-DOPA on mesencephalic cell cultures: protection with antioxidants," *Brain Research*, vol. 682, no. 1-2, pp. 133–143, 1995.

[129] G. Walkinshaw and C. M. Waters, "Induction of apoptosis in catecholaminergic PC12 cells by L-DOPA. Implications for the treatment of Parkinson's disease," *Journal of Clinical Investigation*, vol. 95, no. 6, pp. 2458–2464, 1995.

[130] S. C. Kumer and K. E. Vrana, "Intricate regulation of tyrosine hydroxylase activity and gene expression," *Journal of Neurochemistry*, vol. 67, no. 2, pp. 443–462, 1996.

[131] M. D. De Bellis, A. S. Baum, B. Birmaher et al., "Developmental traumatology—part I: biological stress systems," *Biological Psychiatry*, vol. 45, no. 10, pp. 1259–1270, 1999.

[132] S. T. Kim, J. H. Choi, J. W. Chang, S. W. Kim, and O. Hwang, "Immobilization stress causes increases in tetrahydrobiopterin, dopamine, and neuromelanin and oxidative damage in the nigrostriatal system," *Journal of Neurochemistry*, vol. 95, no. 1, pp. 89–98, 2005.

[133] R. G. Pendleton, F. Parvez, M. Sayed, and R. Hillman, "Effects of pharmacological agents upon a transgenic model of Parkinson's disease in Drosophila melanogaster," *Journal of Pharmacology and Experimental Therapeutics*, vol. 300, no. 1, pp. 91–96, 2002.

[134] P. K. Auluck and N. M. Bonini, "Pharmacological prevention of Parkinson disease in Drosophila," *Nature Medicine*, vol. 8, no. 11, pp. 1185–1186, 2002.

[135] J. Long, H. Gao, L. Sun, J. Liu, and X. Zhao-Wilson, "Grape extract protects mitochondria from oxidative damage and improves locomotor dysfunction and extends lifespan in a drosophila parkinson's disease model," *Rejuvenation Research*, vol. 12, no. 5, pp. 321–331, 2009.

[136] M. Jimenez-Del-Rio, C. Guzman-Martinez, and C. Velez-Pardo, "The effects of polyphenols on survival and locomotor activity in drosophila melanogaster exposed to iron and paraquat," *Neurochemical Research*, vol. 35, no. 2, pp. 227–238, 2010.

[137] R. Hosamani and . Muralidhara, "Prophylactic treatment with Bacopa monnieri leaf powder mitigates paraquatin-duced oxidative perturbations and lethality in Drosophila melanogaster," *Indian Journal of Biochemistry and Biophysics*, vol. 47, no. 2, pp. 75–82, 2010.

[138] K. Faust, S. Gehrke, Y. Yang, L. Yang, M. F. Beal, and B. Lu, "Neuroprotective effects of compounds with antioxidant and anti-inflammatory properties in a Drosophila model of Parkinson's disease," *BMC Neuroscience*, vol. 10, article 1471, p. 109, 2009.

[139] N. Saini and W. Schaffner, "Zinc supplement greatly improves the condition of *parkin* mutant Drosophila," *Biological Chemistry*, vol. 391, no. 5, pp. 513–518, 2010.

[140] G. J. Brunn, C. C. Hudson, A. Sekulić et al., "Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin," *Science*, vol. 277, no. 5322, pp. 99–101, 1997.

[141] P. E. Burnett, R. K. Barrow, N. A. Cohen, S. H. Snyder, and D. M. Sabatini, "RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 4, pp. 1432–1437, 1998.