Parkinson's Disease Model

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. It is known that there are many factors, either genetic or environmental factors, involved in PD, but the mechanism of PD is still not fully understood. Several animal models have been established to study the mechanisms of PD. Among these models, *Drosophila melanogaster* has been utilized as a valuable model to get insight into important features of PD. *Drosophila melanogaster* possesses a well-developed dopaminergic (DA) neuron system which is known to play an important role in PD pathogenesis. The well understanding of DA neurons from early larval through adult stage makes *Drosophila* as a powerful model for investigating the progressive neurodegeneration in PD. Besides, the short life cycle of *Drosophila melanogaster* serves an advantage in studying epidemiological features of PD. Most of PD symptoms can be mimicked in *Drosophila* model such as progressive impairment in locomotion, DA neuron degeneration, and some other non-motor symptoms. The *Drosophila* models of PD, therefore, show a great potential in application for PD genetic and drug screening.

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Keywords

Drosophila melanogaster · Parkinson's disease · PD-like symptoms · Drug screening · Genetic screening

4.1 Introduction

Parkinson's disease (PD) which is characterized by progressive impairment in locomotive ability such as tremor, rigidity, and bradykinesia was first described in 1817 by Dr. James Parkinson. PD impacts 1% of the population over 60 years old and is considered as the second most common neurodegenerative disorder after Alzheimer's disease. Previous studies have shown that PD resulted from the loss of DA neurons in substantia nigra and Lewy body formation in brains (Nussbaum and Polymeropoulos 1997; Forno 1996; Thomas and Beal 2007). Many genes and their variants have been demonstrated to be involved in PD such as *α-synuclein* (*PARK1/SNCA*); *leucine-rich repeat kinase 2* (*PARK8/LRRK2*); *parkin RBR E3 ubiquitin protein ligase* (*PARK2/PARKIN*); *Parkinson protein 7* (*PARK7/DJ-1*); *PTEN-induced putative kinase 1* (*PARK6/PINK1*); *glucosidase*, *beta*, *acid* (*GBA*); and *ubiquitin carboxyl-terminal esterase L1* (*PARK5/UCH-L1*) (Polymeropoulos et al. 1997; Seidel et al. 2010; Paisán-Ruíz et al. 2004; Zimprich et al. 2004; Di Fonzo et al. 2005; Kitada et al. 1998; Hoenicka et al. 2002; Bonifati et al. 2003;

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Annesi et al. 2005; Valente et al. 2004; Hedrich et al. 2006; Aharon-Peretz et al. 2004; Sidransky et al. 2009; Leroy et al. 1998; Liu et al. 2002). Besides, several environmental factors are discovered as causes of PD or to be associated with PD including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), rotenone, and paraquat. In addition, exposure to pesticides or heavy metal, well water consumption, and poor working conditions have been implicated as factors increasing the risk of PD (Pezzoli and Cereda 2013; Montgomery 1995). A variable range of genetic and environmental interaction is also thought to result in PD (Ross and Smith 2007). However, mechanism which causes PD is still unclear. In order to understand PD, some toxin-based models and gene-based models were established. Among those models, *Drosophila melanogaster* have successfully provided valuable insights into the PD (Tieu 2011; Lim and Ng 2009; Dawson et al. 2010; Jagmag et al. 2016).

Drosophila melanogaster has been recognized as a powerful organism for modeling human neurodegenerative diseases including PD. Firstly, many PD-related genes are found to have homologues in *Drosophila*. Secondly, in *Drosophila melanogaster*, most of DA neurons are generated at embryogenesis, matured and gathered into clusters during first larval stage. In adult flies, nine DA neuron clusters can be distinctively recognized by the position of cell body, dendrite, and the number of DA neuron in each cluster. The feature of *Drosophila* is appropriate for applying *Drosophila* PD models in studying the progressive degeneration of neurons (Blanco et al. 2011; Budnik and White 1988). Together with strong points of shortness in life span, large number of population, and easiness in maintenance, the use of *Drosophila* model for PD study has various advantages in genetic analysis in vivo, generation-population analysis.

4.2 Parkinson's Disease and Models for Studying Parkinson's Disease

4.2.1 Parkinson's Disease

Parkinson's disease (PD), a disorder of the basal ganglia, is recognized as one of the most common neurologic disorders, affecting approximately 1% of individuals older than 60 years old. There are two major neuropathologic findings in PD: the loss of pigmented dopaminergic neurons in the substantia nigra and the presence of Lewy bodies. Most cases of idiopathic Parkinson's disease (IPD) are believed to be due to a combination of genetic and environmental factors. The prevalence of PD is about 0.3% of the whole population in industrialized countries. PD is more common in the elderly, and prevalence rises from 1% in those over 60 years of age to 4% of the population over 80. Although 5–10% of cases, classified as young onset, begin between the ages of 20 and 50, the mean age of onset is around 60 years. Some studies have proposed that it is more common in men than women, but others failed to detect any differences between the two sexes. The incidence of PD is between 8 and 18 per 100,000 person-years (Nussbaum and Polymeropoulos 1997; Thomas and Beal 2007; de Lau and Breteler 2006).

In the brain, dopamine plays an important role in controlling muscle activity. When the levels between dopamine and acetylcholine are equal, damping effect occurred in which the basal ganglia will transmit signals to spinal cord to control muscle activity. However, in the PD patients, it is found that dopamine is not produced. Consequently, levels of dopamine and acetylcholine are imbalance, and damping effect has not occurred. Therefore, muscle could not be controlled and resulted in muscle tension and/or tremor (Mayes-Burnett 2016).

Misfolded proteins are known to involve in Parkinson's disease. Misfolded α-synuclein (SNCA), ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), parkin, PTEN-induced putative kinase 1 (PINK1), leucine-rich repeat kinase 2 (LRRK2 or dardarin), and DJ-1 caused overloading the ubiquitin (proteasomal) and lysosomal degradation pathways, thereby resulted in neurodegeneration and PD (Tan et al. 2009; Lee and Hsu 2017).

On the other hand, genetic factors related to oxidative stress, mitochondrial dysfunction, accumulation of α-synuclein, or defects in the ubiquitin-proteasome system are also known to involve in PD (Shadrina et al. 2010). Mutations in specific genes have been conclusively shown to cause PD. In most cases, people with these mutations will develop PD. For example, defects in parkin, UCH-L1, and α-synuclein proteins lead to an error in the protein degradation pathway and caused neurodegeneration. Mutant proteins, such as parkin and UCH-L1, which belong to the ubiquitin-proteasome system, may no longer exert their ubiquitin ligase activity, thus damaging the ability of the cellular machinery to detect and degrade misfolded proteins. PINK1, parkin, and DJ-1 play important roles in maintaining the normal function of mitochondria; therefore mutations in these proteins can result in mitochondrial dysfunction (Ebrahimi-Fakhari et al. 2012; Moon and Paek 2015) (Table 4.1).

Some environmental factors including insecticide, MPTP containing herbicide, rotenone, and paraquat are demonstrated as causes of PD. Besides, air pollution, aging, and working environment are also involved to the high risk of PD (Pezzoli and Cereda 2013; Montgomery 1995). The complex interaction between environmental and genetic factors is also thought to result in PD, but the interlink between these factors still remains unknown.

Table 4.1 Parkinson's disease-related proteins

Protein	Organ/functional system
α-Synuclein	Mitochondria, ubiquitin-proteasome system
Parkin	Mitochondria, ubiquitin-proteasome system
UCH-L1	Ubiquitin proteasome system
PINK1	Mitochondria
DJ-1	Mitochondria, ubiquitin-proteasome system
LRRK2	Mitochondria
HtrA2	Mitochondria
GBA	Lysosome
POLG	Mitochondria

Although many genes, proteins, and environmental factors are known to be involved in PD, the mechanism of this disease is still unclear, leading to many limitations in studying and finding PD drugs. In order to find out a therapy for PD, recently, there are many researches focus on mechanism of PD which is based on Lewy body, oxidative stress, mitochondria, and ubiquitin-proteasome system.

4.2.2 Models for Studying Parkinson's Disease

To study on Parkinson's disease, many models have been established and utilized. The models of PD can be divided into two different approaches: toxin-based models (such as 6-OHDA, MPTP, rotenone, and paraquat) and gene-based models (such as α-synuclein, LRRK2, Parkin, DJ-1, PINK1) (Tieu 2011; Lim and Ng 2009; Dawson et al. 2010; Jagmag et al. 2016; Dauer and Przedborski 2003; Hisahara and Shimohama 2011). Many cellular and animal models of PD have been developed to investigate the mechanism of PD and develop new therapeutic strategies. An ideal model of PD should display pathophysiologic features and symptoms of PD; however, the current models are not able to recapitulate all PD features. Each model has both advantages and disadvantages, and the selection of the most suitable model depends on particular purposes of the research study (Fig. 4.1).

4.2.2.1 Cellular Models

Cellular models have been used for studying PD mechanism, drug screening, and developing new therapeutic strategies. In addition to the strengths of cell-based model including easy access of cells in culture and allowing high-throughput screening, PD cellular models can display fea-

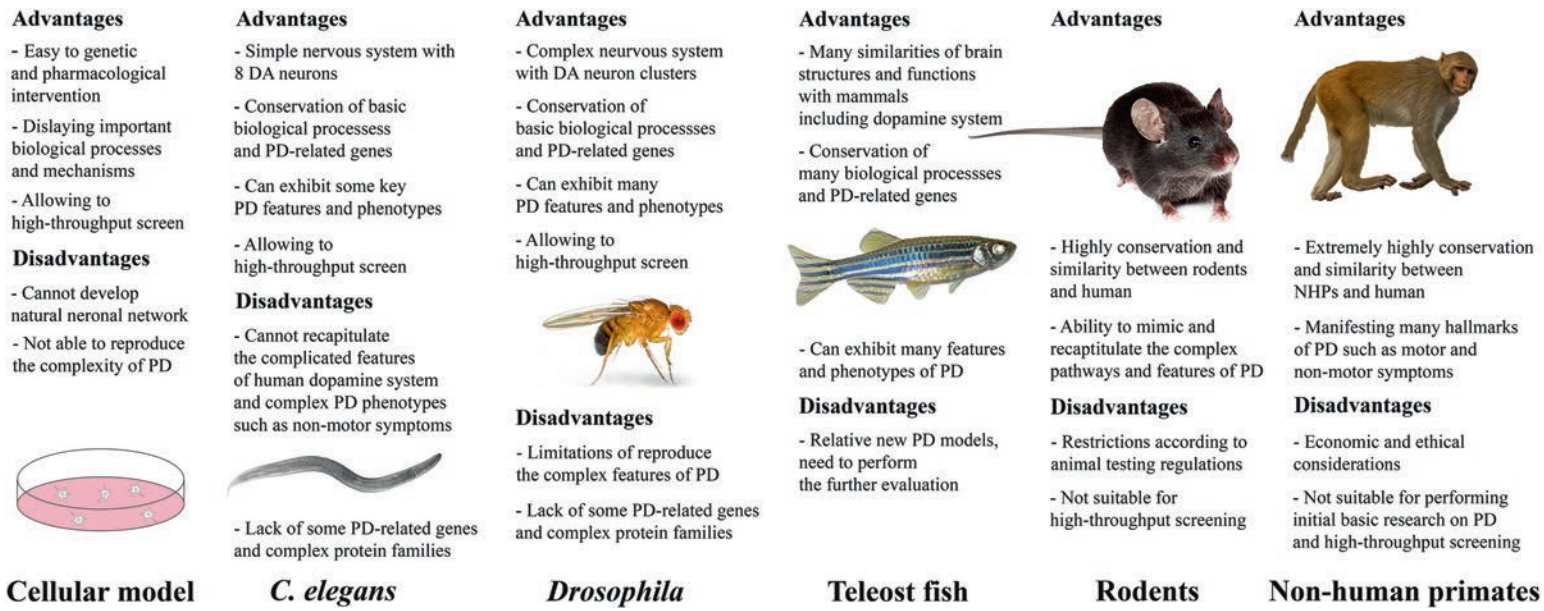

Fig. 4.1 Animal models in studying Parkinson's disease: advantages and disadvantages

tures of PD (such as DA neuron degeneration and protein aggregates containing α-synuclein) and important biological processes (such as apoptosis, oxidative stress, mitochondrial impairment, altered proteolysis, and dysfunctional mitophagy) (Alberio et al. 2012; Falkenburger and Schulz 2006; Falkenburger et al. 2016). However, the weaknesses of cellular models are that culture cells do not develop natural neuronal network and lack the interaction of different cell types and cellular microenvironment; therefore they are not able to reproduce the complexity of PD (Falkenburger and Schulz 2006).

Human neuroblastoma cell line SH-SY5Y and rat pheochromocytoma cell line PC12 are cell lines widely used for modeling PD. They possess the machinery to produce and release catecholamines and can develop neuron-like features. Numerous studies have used these cell lines as PD models to not only screen causative factors that can cause PD as well as compounds that can treat PD but also study the molecular and cellular mechanism related to PD (Xicoy et al. 2017; Malagelada and Greene 2008). Besides that, immortalized lund human mesencephalic (LUHMES) cells can be used for modeling PD because of their ability to differentiate and develop to dopaminergic-like neurons (Zhang et al. 2014). Another approach to model PD is using patient-specific cell lines (Schule et al. 2009). Cybrid (cytoplasmic hybrid) cell lines are created by fusion of mtDNA-lacking cell and donated platelets containing mtDNA from PD patients. The PD cybrid cell lines can represent the impairment in mitochondrial functions and have been used to investigate the relationship between mtDNA gene mutation and mitochondrial dysfunction and PD pathogenesis (Trimmer and Bennett 2009). Recently, the development of human-induced pluripotent stem cells (iPSCs) has supported the studies of human diseases including PD. The abilities to derive iPSCs from PD patients and differentiate these iPSCs into DA neurons exhibiting PD phenotypes enable them to become a promising model to study mechanism and drug discovery (Martinez-Morales and Liste 2012; Byers et al. 2012).

4.2.2.2 Animal Models

There are numerous animal models that have been developed from invertebrates such as nematode roundworm and fruit fly to vertebrates including fish, rodent, and nonhuman primates. The uses of these models have been significantly contributed to our knowledge of PD pathogenesis and potential treatment.

4.2.2.2.1 Nematode Roundworm: *Caenorhabditis elegans*

C. elegans possesses many advantages of modeling PD for studying the complex interaction of genetic and environmental factors and drug screening. This simple organism shares many conserved molecular and cellular pathways to human such as protein degradation machinery, oxidative stress, and signal transduction (Harrington et al. 2010). Specially, *C. elegans* has simple nervous system with exactly 302 neurons including 8 dopaminergic neurons and the conserved dopaminergic pathways (Harrington et al. 2010; Sulston et al. 1975). Although the simple dopaminergic system is useful to study the effects of factors on morphology and number of DA neurons, it cannot recapitulate the complex features of human dopamine neurons.

The *C. elegans* genome encodes many homologues of PD-related genes such as Parkin, PINK1, DJ-1, UCH-L1, and LRRK2, so this organism can be used for studying the functions of these genes involved in PD. For example, the study on Lrk-1, a homolog of LRRK2 in *C. elegans*, demonstrated the role of this protein in regulating cellular responses to mitochondrial dysfunction (Saha et al. 2009). Although there is an absence of the *C. elegans* α-synuclein homolog, transgenic roundworm model which overexpresses human α-synuclein has been developed. The *C. elegans* model established by Lakso et al. showed that the overexpression of α-synuclein in DA neurons led to neurodegeneration (Lakso et al. 2003). Remarkably, a whole genome microarray analysis on α-synuclein-overexpressing *C. elegans* was performed to identify gene expression changes. That supported confirmation of known molecular functions and suggestion of

new pathways related to PD and contributed to understand the role of α-synuclein in PD pathogenesis (Vartiainen et al. 2006). Neurotoxins such as 6-OHDA and MPP+ are also used to develop *C. elegans* models of PD (Li and Le 2013). In addition to study PD mechanism, *C. elegans* is also a suitable model for drug discovery (Chen et al. 2015).

4.2.2.2.2 Fruit Fly: *Drosophila melanogaster*

The completion of the genome sequence showed that 77% of human disease genes are conserved in *Drosophila (*Adams et al. 2000*)*. Notably, many homologues of PD-related genes were identified in fruit fly such as dardarin/LRRK2, parkin, PINK1, Omi/HtrA2, DJ-1, UCH-L1, GIGYF2, PLA2G6, and GBA with exception of α-synuclein, ATP13A2, and FBXO7 (Whitworth 2011). *Drosophila* possesses more complex dopaminergic neuron system containing DA neuron clusters. In larval stage, there are 21 DA neurons grouped into 7 DA neuron clusters per hemisphere: DM1a, DM1b, DM2, DL1a, DL1b, DL2a, and DL2b (Blanco et al. 2011). In adult stage, DA neurons are classified into nine distinct clusters: PAM, PAL, PPM1, PPM2, PPM3, PPL1, PPL2ab, PPL2c, and VUM (Fig. 4.2) (Nassel and Elekes 1992; Mao and Davis 2009). The locations of DA neuron clusters have been identified; the effects of environmental or genetic factors on the number, morphology, or locations of DA neurons can be examined. Moreover, beside the similarity in some main functions of nervous system between human and fly, the basic biological processes such as cell death regulation are also conserved in *Drosophila (*Jennings 2011; Vernooy et al. 2000*)*. Considering these strengths, *Drosophila* is a powerful tool for study of PD.

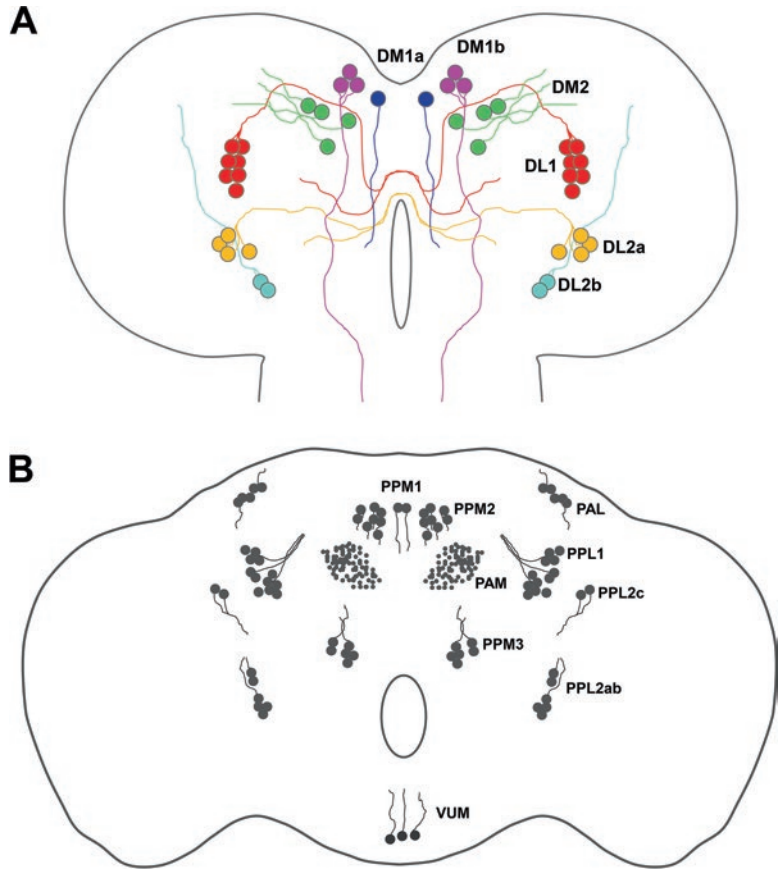

Fig. 4.2 Schematic representation of DA neuron systems in the *Drosophila* larval and adult stages. (**a**) Illustration of six DA neuron clusters DM1a, DM1b, DM2, DL1, DL2a, and DL2b in *Drosophila* larval central brain. The illustration was redrawn based on the study of Blanco et al. (Blanco et al. 2011). (**b**) A schematic representation of seven DA neuron clusters PAL, PAM, PPM1, PPM2, PPM3, PPL1, and PPL2 in *Drosophila* adult central brain. It was redrawn based on the study of Nassel et al. and Mao et al. (Nassel and Elekes 1992; Mao and Davis 2009)

4.2.2.2.3 Teleost Fish: Zebrafish and Medaka Fish

Teleost fish including zebrafish and medaka fish has been widely used as model for studying developmental biology and recently emerged as a new vertebrate model of PD (Matsui and Takahashi 2017). These fish have several strengths such as transparency high fecundity, their rapid development, and ease of maintenance and handling (Xi et al. 2011; Matsui et al. 2012). Notably, teleost fish is a vertebrate, so these fish possess many similarities of brain structures and functions with mammals including dopamine system. DA neuron clusters (A8–A10) in the diencephalon-midbrain are closely related to PD (German et al. 1989). Although teleost midbrain does not contain DA neurons, they are located in the paraventricular organs, the periventricular nucleus of the posterior tuberculum, and the posterior tuberal nucleus. Within these DA neurons, some neurons in the periventricular nucleus of the posterior tuberculum may be equivalent to mammalian A9 and A10 neurons because of their projection pattern (Matsui 2017).

There are several zebrafish and medaka fish models of PD induced by genetic (parkin, PINK1, DJ-1, LRRK2, ATP13A2, and GBA) or toxin factors (MPTP and 6-OHDA). Several fish models exhibited some features of PD including reduction of locomotive ability (swimming movement) and loss of DA neurons (Xi et al. 2011; Matsui et al. 2012). These models have been used for studying the contributions of lysosome dysfunction and mitochondrial dysfunction to PD (Matsui and Takahashi 2017). Recently, Zhang et al. developed zebrafish model combining PINK1 deficiency and rotenone for drug screening (Zhang et al. 2017). However, these teleost fish are relative new PD models; therefore, the further evaluation of these organisms as PD models needs to perform.

4.2.2.2.4 Mammals: Rodent and Nonhuman Primate

The highly conservation and similarity between mammals including rodent and nonhuman primates (NHP) and human make these organisms as good PD models. Rodent and NHP models are expected to exhibit complex features of PD and closely match to human pathology. Similar to abovementioned models, PD rodent models can be classified into environmental models, induced by several neurotoxins such as MPTP and 6-OHDA and genetic models with knock-in or knockout of PD-related genes. These models have provided insight into pathways involved in PD and contributed to therapeutic development (Vingill et al. 2017). The well-established NHP model of PD is induced by MPTP and manifests many hallmarks of PD including DA cell loss and motor and non-motor symptoms such as cognitive impairment and sleep/wake disturbances (Porras et al. 2012). Recently, another approach to model PD NHP model is using AAV1/2 vector to overexpress α-synuclein; however, this methodology is relatively new and needs extended study (Koprich et al. 2016). NHP model has been used as a preclinical model of PD and plays an important role in developing treatment therapies for PD (Blesa et al. 2017). However, the use of these mammalian models is limited by economic and ethical considerations, and these models are not suitable for performing initial research on PD because of their complexities.

4.3 *Drosophila* Model in Studying Parkinson's Disease

4.3.1 *Drosophila* Models of Parkinson's Disease

Many *Drosophila* models of Parkinson's disease based on pathogenic molecular mechanisms have been developed, either by gene transfer or by induction with poison. Fly models have been reported to exhibit strong PD-like phenotypes characterized by locomotion defects and DA neuron degeneration as well as defects associated with mitochondrial dysfunction, oxidative stress, and protein aggregation (Whitworth 2011). Many *Drosophila* models of PD induced by genetic factors including α-synuclein, LRRK2, Parkin, DJ-1, and PINK1 and environmental factors such as rotenone and paraquat have been developed, and studies on these models provided some profound insights into PD pathogenesis (Whitworth 2011; Navarro et al. 2014) (Table 4.2). For instance, research on *Drosophila* has clarified the functions

Table 4.2 *Drosophila* models of Parkinson's diseases

Toxin-based models of PD						
Toxin			PD-like phenotypes			Relevant biological processes
			Locomotive defects	LB-like aggregations	Loss of DA neurons	
Rotenone			Yes (Coulom and Birman 2004)	No (Coulom and Birman 2004)	Yes (Coulom and Birman 2004)	Mitochondrial oxidative stress (Hosamani et al. 2010), and the mitochondrial fusion/fission machinery (Hwang et al. 2014)
Paraquat			Yes (Ameel et al. 2007)	No data	Yes (Ameel et al. 2007)	Oxidative stress, mitochondrial dysfunction (Shukla et al. 2016; Hosamani 2013), and DNA damage (Mehdi and Qamar 2013)
Genetic-based models of PD						
Gene	*Drosophila* homolog (identity)	Genetic intervention	PD-like phenotypes			Relevant biological processes
			Locomotive defects	LB-like aggregations	Loss of DA neurons	
SNCA	No	Expression of human WT/ A30P/ A53T	Yes (Feany and Bender 2000)	Yes (Feany and Bender 2000)	Yes (Feany and Bender 2000)	Lipid metabolism, energy production, membrane transport (Scherzer et al. 2003), and oxidative stress (Botella et al. 2008; Trinh et al. 2008)
		Expression of S129D	No data	Yes (Chen and Feany 2005)	Yes (Chen and Feany 2005)	
		Expression of WT 1–120 construct	No data	Yes (Periquet et al. 2007)	Yes (Periquet et al. 2007)	
LRRK2	dLRRK (26%) (Whitworth 2011)	Expression of human WT/ G2019S	Yes (Liu et al. 2008)	No data	Yes (Liu et al. 2008)	Oxidative stress, protein translation (Imai et al. 2008), energy demand (Hindle et al. 2013), vesicular transport (Dodson et al. 2012, 2014; Arranz et al. 2015; Linhart et al. 2014), and cytoskeleton regulation (Lee et al. 2010)
		Expression of human R1441C	Yes (Islam et al. 2016)	No data	Yes (Islam et al. 2016)	
		dLRRK null mutant	Yes (Lee et al. 2007)	No data	No (Lee et al. 2007; Wang et al. 2008)	
Parkin	Parkin (42%) (Whitworth 2011)	Expression of human Q311X/T240R	Yes (Sang et al. 2007)	No data	Yes (Sang et al. 2007)	Mitochondrial dysfunction, apoptosis (Greene et al. 2003), mitochondrial fusion/ fission machinery (Deng et al. 2008), oxidative stress, innate immune responses (Greene et al. 2005; Whitworth et al. 2005), and ER stress (Celardo et al. 2016)
		Parkin null mutant	Yes (Whitworth et al. 2005)	No data	Yes (Whitworth et al. 2005)	

(continued)

Table 4.2 (continued)

DJ-1	DJ-1α (56%) (Whitworth 2011)	DJ-1α null mutant	No data	No data	No (Meulener et al. 2005)	Oxidative stress, apoptosis (Yang et al. 2005; Hwang et al. 2013), and mitochondrial dysfunction (Hao et al. 2010)
		Knockdown of DJ-1α by Ddc-Gal4, TH-Gal4, and Elav-Gal4	No data	No data	Yes (Yang et al. 2005)	
	DJ-1β (52%) (Whitworth 2011)	DJ-1β null mutant	Yes (Park et al. 2005; Lavara-Culebras and Paricio 2007)	No data	No (Park et al. 2005; Lavara-Culebras and Paricio 2007)	
PINK1	PINK1 (32%) (Whitworth 2011)	PINK1 null mutant	Yes (Park et al. 2006)	No data	Yes (Park et al. 2006)	Mitochondrial dysfunction (Park et al. 2006) and mitochondrial fusion/fission machinery (Yang et al. 2008)
		Knockdown of PINK1 by Da-Gal4 or TH-Gal4	Yes (Yang et al. 2006)	No data	Yes (Yang et al. 2006)	
GBA	dGBA1a (32%) dGBA1b (31%) (Whitworth 2011)	Double heterozygous dGBA1a and dGBA1b mutant	Yes (Maor et al. 2013)	No (Maor et al. 2016)	Yes (Maor et al. 2016)	ER stress (Maor et al. 2016; Suzuki et al. 2013)
		Expression of human N370S/L444P	Yes (Maor et al. 2013)	No data	Yes (Maor et al. 2016)	
UCH-L1	dUCH (45%) (Whitworth 2011)	Knockdown of dUCH by TH-Gal4	Yes	No data	Yes	Oxidative stress

of PINK1 and parkin which are associated with familial forms of PD. Many studies on fly model were performed and showed that parkin acts as a downstream of PINK1, and this pathway regulates mitochondrial integrity and mitochondrial fission/fusion dynamics (Guo 2010). Besides that, *Drosophila* has been also considered as a model for high-throughput screening of candidate compounds that can prevent this disease and developing therapeutic strategies (Whitworth 2011; Whitworth et al. 2006). Fly with PD symptoms caused by oxidative stress can be used for rapid screening of potential therapeutic antioxidant drugs in treating PD such as melatonin with the paraquat model and polyphenols with the α-synuclein model (Medina-Leendertz et al. 2014; Takahashi et al. 2015).

4.3.2 Parkinson's Disease Symptoms and PD-Like Phenotypes in *Drosophila* Models

The basic symptoms of Parkinson's disease are difficulty in walking, slow movement, stiff and trembling limbs, balance disorders, and facial paralysis. Symptoms appear gradually and not marked, and it is difficult to recognize and often may be confused with other diseases. Causes are attributed to lack of dopamine, a chemical that plays an important role in nerve signal transmission, due to degeneration/loss of dopaminergic neurons. Besides, the presence of Lewy body was also reported as one of the PD symptoms although it is not clear to be a cause or a result of PD

(Nussbaum and Polymeropoulos 1997; Mayes-Burnett 2016; Fahn and Sulzer 2004).

4.3.2.1 PD-Like Phenotype of Movement

In fly model, the progressive impairment in locomotive ability of PD has been characterized through crawling ability in larval stage and climbing ability in adult stage. Many studies on *Drosophila* PD models showed the similarity of locomotor behaviors including decline in climbing ability of *Drosophila* overexpressing human wild-type and PD-related mutant forms of alpha-synuclein, reduction in crawling ability of parkin mutant third instar larvae and locomotor dysfunction, and early mortality in *Drosophila* overexpressing human wild-type and PD-associated mutant forms of LRRK2 (Feany and Bender 2000; Liu et al. 2008; Sang et al. 2007).

The assay to quantify the locomotor ability of *Drosophila* larvae (crawling assay) was first described by Min and Condron in 2005 (Min and Condron 2005). In this assay, larvae in the third instar stage were randomly picked up from PD fly models and placed on agar plate to examine crawling ability. Larval movement was recorded, and then the recorded videos were analyzed to track larval movement and draw motion paths. The average velocity was also calculated, statistically analyzed, and graphed. The PD model larvae displayed a tremor-like behavior which was tracked as tight wavy line when moving horizontally on agar plates. Additionally, these larvae accomplished a shorter moving path compared to normal flies. The mean velocity of PD larvae was also reduced in comparison with the normal flies (Fig. 4.3).

Locomotor ability of adult flies can be estimated by startle-induced negative geotaxis assay which was first described in 1992 by Le Bourg and Lints as climbing activity (Le Bourg and Lints 1992). Flies were transferred from food vials to climbing cylinders and then were tapped to the bottom, and the movement of flies was recorded. The data then were statistically analyzed. The PD model flies showed the decline in climbing ability in comparison with normal flies (Fig. 4.4).

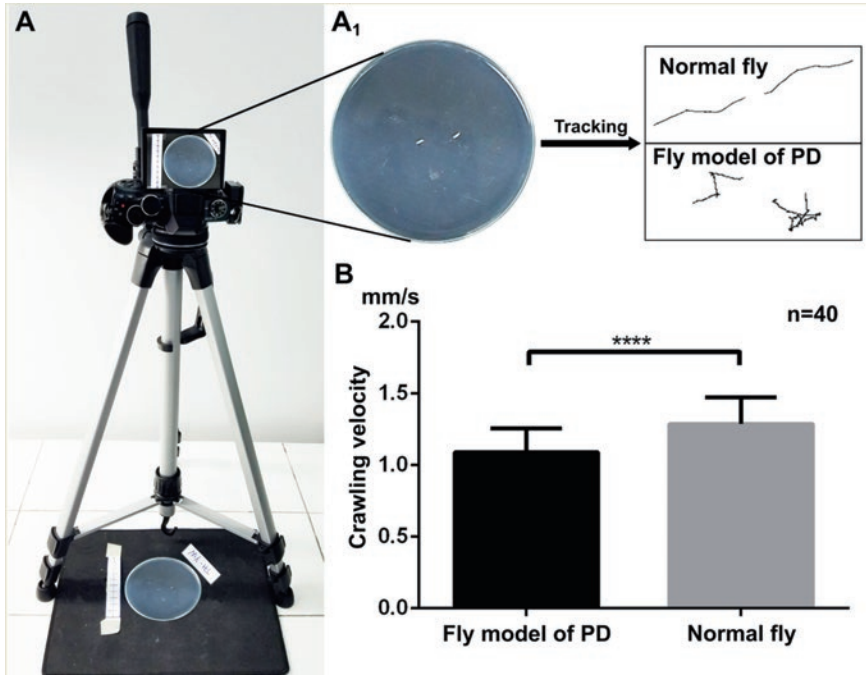

Fig. 4.3 PD-like phenotype of movement in larvae can be scored by crawling assay. (**a**) Larval movement and draw motion path. (**b**) Crawling velocity

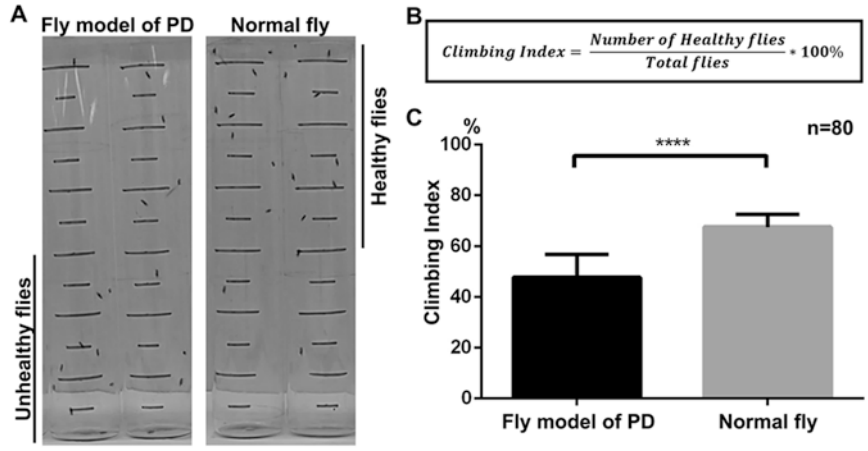

Fig. 4.4 Climbing assay: an acquisition of PD-like phenotype of movement in adult fly. (**a**) Visualization of climbing assay. (**b**) Formula of climbing index. (**c**) A representation of climbing index

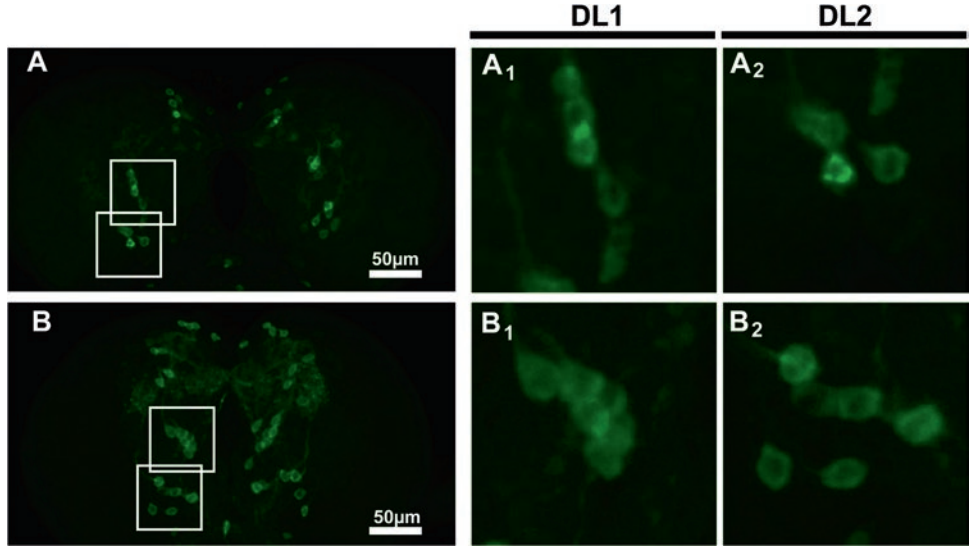

Fig. 4.5 The loss of DL1 and DL2 dopaminergic neurons in larval brain with knockdown of dUCH, a homolog of UCH-L1 in *Drosophila*. DA neuron clusters in the third instar larval central brain were stained with anti-TH. (**a**) PD model larval brain. (**b**) Normal larval brain. The boxed area marks DL1 and DL2 clusters were magnified in A_1 and B_1 and A_2 and B_2. Number of DA neurons in DL1 and DL2 clusters in PD model larval brain was less than those in normal flies

4.3.2.2 PD-Like Phenotype of DA Degeneration

Since *Drosophila* possess a complex dopaminergic neuron system containing DA neuron clusters, fly models can emulate PD symptom of DA loss/degeneration. DA neuron in fly can be visualized by immunostaining with anti-tyrosine hydroxylase (anti-TH), an enzyme that plays a key role in dopamine synthesis pathway. Number of DA in each DA cluster can be examined at both larval and adult stage (Figs. 4.5 and 4.6).

4.3.2.3 PD-Like Phenotype of Aging-Dependent Progression

Parkinson's disease is not only characterized by the degeneration but also by the progressive loss

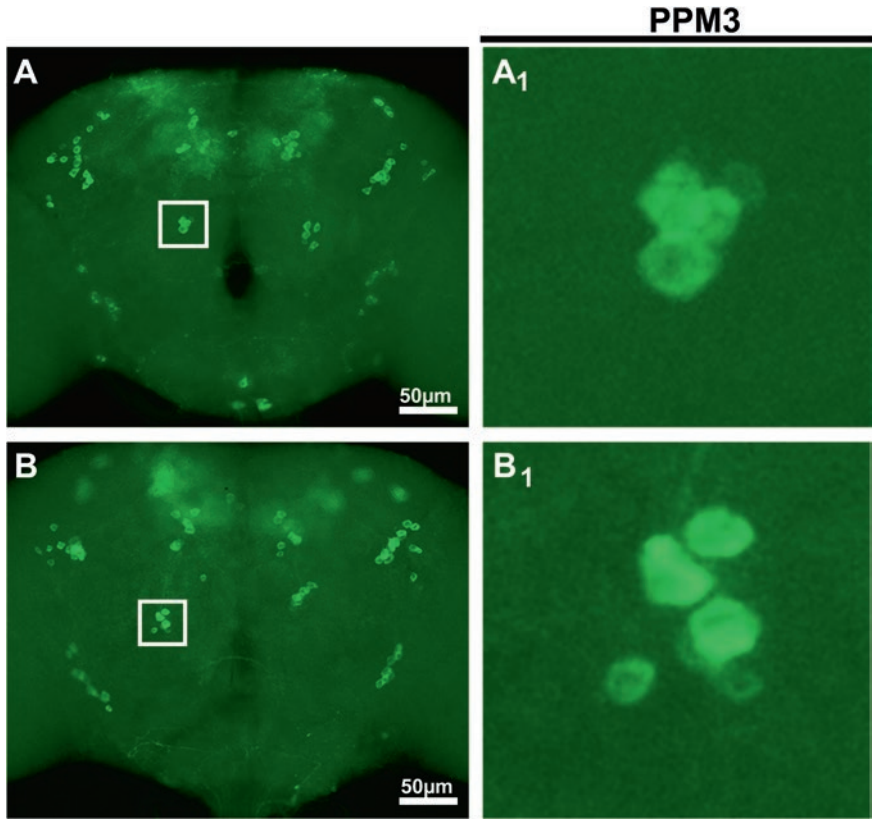

Fig. 4.6 The susceptibility of PPM3 dopaminergic neurons in adult brain of dUCH knockdown fly. DA neuron clusters in fly brain were stained with anti-TH. (**a**) PD model fly brain. (**b**) Normal fly brain. The boxed area marks PPM3 cluster was magnified in A_1 and B_1

of DA neurons in the course of aging. The short life span of *Drosophila* makes it convenient to perform aging-dependent analysis in relatively short time periods. Thereby, *Drosophila* models of PD own a strong point in observing the aging-dependent PD characteristic. The observation of DA neurons in fly brain can be performed from 1-day-old to 40-day-old adult fly brains to see if PD model brains exhibit gradual reduction in the number of DA neurons.

Besides, in the epidemiological point of view, the percentage of individuals with PD in the population increases throughout aging. The most advantage of *Drosophila* models in studying PD is the easiness to handle numerous samples at one time, by which *Drosophila* models can provide reliable data for statistical analysis without bias. Together with a strong point of life span shortness, *Drosophila* serves as a good model for calculating the percentage of flies which showed aberrant DA neuronal phenotype in PD model fly population from 1 to 40 days old. The prevalence can be count in correlation with aging (Fig. 4.7).

4.3.2.4 PD-Like Phenotype of Dopamine Shortage

Reduction of neurotransmitter dopamine was found in PD patients and was declared as PD clinical symptoms (Jankovic 2008). Dopamine is mainly produced in DA neurons through catecholamine biosynthesis pathway. Dopamine in fly brains can be quantified by high-performance liquid chromatography (HPLC). Adult fly heads are collected and homogenized in homogenization buffer containing 0.1 M perchloric acid and 3% trichloroacetic acid. Supernatants of the homogenates are used for performing HPLC. Studies on *Drosophila* model have dem-

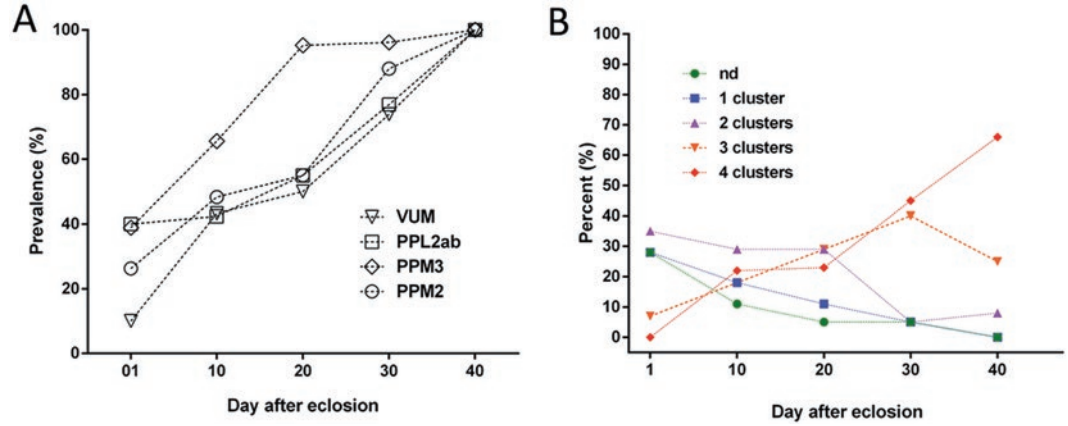

Fig. 4.7 The progressive loss of DA neurons in the course of aging in dUCH knockdown fly brain. (**a**) Prevalence was increased in correlation with aging in VUM, PPL2ab, PPM3, and PPM2 clusters. The prevalence in PPM2 and VUM increased in regular manner from 1 to 40 days old, while the prevalence in PPM3 increased rapidly from 1 to 20 days old and then went to stationary phase from 20 to 40 days old, and the prevalence in PPL2ab slowly increased from 1 to 40 days old. (**b**) The percentage of dUCH knockdown flies with no damage on DA neuron system was 28.6% at 1 day old and decreased regularly from 1 to 40 days old. The similar phenomenon occurred in one and two DA cluster-damaged flies with 28.6% and 35.7%, respectively, at 1 day old, and they also decreased regularly from 1 to 40 days old. In contrast, three and four DA cluster-damaged flies with 7.1% and 0%, respectively, at 1 day old experienced rapid increases from 1 to 30 days old

onstrated the *Drosophila* locomotor activity involved in dopamine level (Riemensperger et al. 2013). In addition, some other *Drosophila* life activities such as olfactory conditioning, sleep and arousal regulation, and memory and learning process also relate to dopamine production (Selcho et al. 2009; Ueno et al. 2012; Berry et al. 2012). Those mentioned activities are known as non-motor features of PD.

4.3.2.5 Lewy Body-Like Aggregation in *Drosophila*

Lewy body (LB), a fibrillar aggregation in brain, has been considered as a histological hallmark of Parkinson's disease (PD). Since neuronal loss is found in predilection sites of LBs, LB formation has been considered as a marker for neurodegeneration. The main component of LB is known to be α-synuclein (α-syn), which is the first protein in which mutants A30P and A53T were found to cause PD. Ectopic expression of human α-syn either wild-type or PD-linked mutants (A53T and A30P) in *Drosophila* mimics some aspects of PD such as locomotion dysfunction, LB accumulation, and neurodegeneration (Feany and Bender 2000; Chen and Feany 2005; Periquet et al. 2007). Furthermore, role of molecular chaperones and protein degradation systems in protecting against α-syn misfolding has been investigated by using α-syn *Drosophila* models (Mizuno et al. 2011).

4.3.2.6 Non-motor PD Phenotypes in *Drosophila*

In addition to impairment in locomotion, Parkinson's disease is also known as a multi-system disorder with non-motor features. Throughout the course of PD, reflecting the neurodegeneration, various clinical symptoms have been observed in PD patients. The symptoms are involved not only in the dopaminergic degeneration but also in damaging of other brainstem areas such as serotonergic, noradrenergic, and cholinergic frontal brainstem (Perez-Lloret and Barrantes 2016). Non-motor symptoms in PD occurred throughout the course of the disease either in early or late stage. In later development of PD, several non-motor symptoms including sleep, smell, and mood problems have been observed. Some symptoms such as sleep and autonomic disturbances occurred diversely in early and later PD stages. Other non-motor features are also found in de novo, untreated PD

patients such as cognitive impairment and autonomic dysfunction (Perez-Lloret and Barrantes 2016; Goldman and Postuma 2014). In *Drosophila*, modeling of PINK1 and parkin loss-of-function mimic a range of non-motor PD features. Abnormalities in learning and memory were recorded in both Pink1 and Parkin *Drosophila* models of PD. Besides, weakness of circadian rhythm was also observed (Julienne et al. 2017). The *Drosophila* model of PD therefore showed its advantage in studying PD with non-motor phenotypes.

4.4 *Drosophila* Model of Parkinson's Disease and Applications

4.4.1 The Contributions of *Drosophila* to Study PD

After Feany and Bender established the first *Drosophila* model of PD by expressing normal and mutant forms of human α-synuclein in 2000 (Feany and Bender 2000), numerous *Drosophila* models have been developed induced by both environmental and genetic factors for studying PD. Research on *Drosophila* has provided several important insights into PD pathogenesis. One of the outstanding contributions of fly model is elucidating the endogenous functions of PINK1 and parkin from studies on *Drosophila* homologues of these genes. The studies on fly model have provided the strong evidence that PINK1 and parkin function in regulating mitochondrial integrity. Flies with null mutants in parkin manifest locomotive impairment, mitochondrial defects, and DA neuron degeneration (Greene et al. 2003; Whitworth et al. 2005; Pesah et al. 2004). Subsequent studies showed that PINK1 mutants resulted in phenotypes similar to parkin mutants including mitochondrial dysfunction. Furthermore, overexpression of parkin can suppress the phenotypes induced by PINK1 mutant, whereas PINK1 overexpression cannot rescue parkin mutant phenotypes. The data indicated that Parkin functions downstream of PINK1 in a common pathway for maintaining mitochondrial integrity (Park et al. 2006; Yang et al. 2006; Clark et al. 2006). Notably, these findings on *Drosophila* model are consistent with human and mice. Cells from PD patient with parkin or PINK1 mutants and human cell with knockdown of PINK1 showed defects in mitochondrial morphology and functions (Muftuoglu et al. 2004; Grunewald et al. 2010; Gegg et al. 2009; Exner et al. 2007). The observations in mouse models indicated that knockout of PINK1 or parkin also caused impairments in mitochondrial respiration but not morphology (Palacino et al. 2004; Gautier et al. 2008). Moreover, aberrant mitochondrial morphology in PINK1 knockdown cell was rescued by expression of parkin (Exner et al. 2007).

The further investigations on *Drosophila* showed that PINK1 and parkin play important roles in mitochondrial dynamics and mitophagy. Several studies indicated that PINK1 and parkin interact with regulators of fusion/fission machinery. The phenotypes of parkin or PINK1 mutants such as defects in locomotive abilities and mitochondrial morphology were suppressed by overexpression of fission factor drp1 (dynamin-related protein 1) or reduction of fusion factors mfn (mitofusin) and opa1 (optic atrophy 1) (Deng et al. 2008; Yang et al. 2008; Poole et al. 2008; Park et al. 2009). The data show that PINK1/parkin pathway promotes mitochondrial fission and/or inhibits fusion. Subsequently, Parkin was demonstrated to induce the ubiquitination of Mfn in fly models (Poole et al. 2010; Ziviani et al. 2010) and mammalian cells (Tanaka et al. 2010). Moreover, PINK1/parkin pathway also promotes mitophagy. A study in fly model using proteomic approach showed that parkin null mutants slowed the mitochondrial protein turnover and PINK1 mutants resulted in selective impairment in mitochondrial respiratory chain subunit turnover. The study on *Drosophila* model of PD provides the evidence of the function of PINK1/parkin pathway in mitophagy (Vincow et al. 2013).

In addition to studying functions of PINK1 and parkin, *Drosophila* model also provided key insights into the relationship between other genetic and environmental factors and biological processes, as well as the interaction of these factors. For example, *Drosophila* models of PD

induced by toxins showed that rotenone toxicity is related to mitochondrial oxidative stress (Hosamani et al. 2010) and the mitochondrial fusion/fission machinery (Hwang et al. 2014). In PD fly models induced by genetic factors, several studies indicated that dLRRK/LRRK2 is involved in processes including oxidative stress, protein translation (Imai et al. 2008), energy demand (Hindle et al. 2013), vesicular transport (Dodson et al. 2012, 2014; Arranz et al. 2015; Linhart et al. 2014), and cytoskeleton regulation (Lee et al. 2010). Another PD-related gene, dDJ-1/DJ-1, was reported to play roles in oxidative stress response, apoptosis (Yang et al. 2005; Hwang et al. 2013), and mitochondrial function (Hao et al. 2010). Moreover, the sensitivity of dDJ-1 mutant flies to oxidative stress-inducing toxin exposure suggested that dDJ-1 play a role in the protection from environment oxidative stress and provided a link between genetic and environmental factors in PD pathogenesis (Meulener et al. 2005). In other studies, dDJ-1 knockout flies exhibited mitochondrial defects, and upregulation of dDJ-1 can rescue muscle defects caused by PINK1, but not parkin, mutants. The results obtained in this study suggested complex interaction between DJ-1 and PINK1/parkin pathway (Hao et al. 2010).

Previous studies implicated mitochondrial dysfunction, oxidative stress, altered proteolysis, and inflammation in the pathogenesis of PD (Shadrina et al. 2010; Dexter and Jenner 2013; Klemann et al. 2017). The complex interaction between environmental and genetic factors is considered to result in PD; however, the roles of these factors as well as the interactions between them leading to this disease have not yet been elucidated in detail. The findings in *Drosophila* model contribute to our knowledge about PD pathogenesis.

4.4.2 The Applications of *Drosophila* to Genetic and Drug Screening

Drosophila possesses many useful features such as short life cycle, available genetic tools for manipulation of genome, and the conservation of basic biological processes and PD-related genes. Besides that, many key neuropathologic and clinical features of PD are reproduced in fly model. Therefore, *Drosophila* is also considered as a powerful tool for genetic and drug screening. The genetic screens allow genomic-wide analysis of genetic interactions to identify genes that can enhance or suppress the phenotypes caused by a mutant gene of interest (Fig. 4.8). For instance, *Drosophila* was used in a genome-wide screening project for modifiers parkin and PINK1 mutant phenotypes. In the study, flies with knockdown of parkin or PINK1 and PINK1 null mutant were crossed with deficiency lines, and analysis of wing phenotype, longevity, and fertility was performed. By analyzing cytological regions interacting with parkin and/or PINK1, five candidate genes were identified including opa1, drp1, dbr, Pi3K21B, and β4GalNAcTA (Fernandes and Rao 2011). Another study identified acon (aconitase) as a dominant suppressor of PINK1 by performing a genetic modifier screening in PINK1 mutant fly model (Esposito et al. 2013).

In the field of compound screening, there are two distinct approaches. The first approach is screening toxins that can induce abnormal phenotypes in wild-type flies. The second approach is testing drug that can rescue aberrant phenotypes induced by mutation, RNAi, transgenesis, or chemical (Giacomotto and Ségalat 2010). Drug screening on *Drosophila* model helps to discover potential therapeutic compounds for PD (Fig. 4.9). For example, dDJ-1β mutant fly was used for performing modifier compound screen. This study identified candidate chemicals such as dexrazoxane, tocopherol, sodium phenylbutyrate, dalfampridine, methylene blue, and minocycline that are able to improve climbing ability. Furthermore, these positive candidate compounds also attenuate H2O2-induced cytotoxicity of DJ-1 mutant human cells (Sanz et al. 2017). In another study, *Drosophila* expressing human mutant LRRK2 (G2019S) was utilized to validate seven phenolic compounds which show kinase inhibitor activity. The results showed that piceatannol, thymoquinone, and esculetin reduced oxidative stress and the loss of DA neurons and locomotor defects caused by expressing

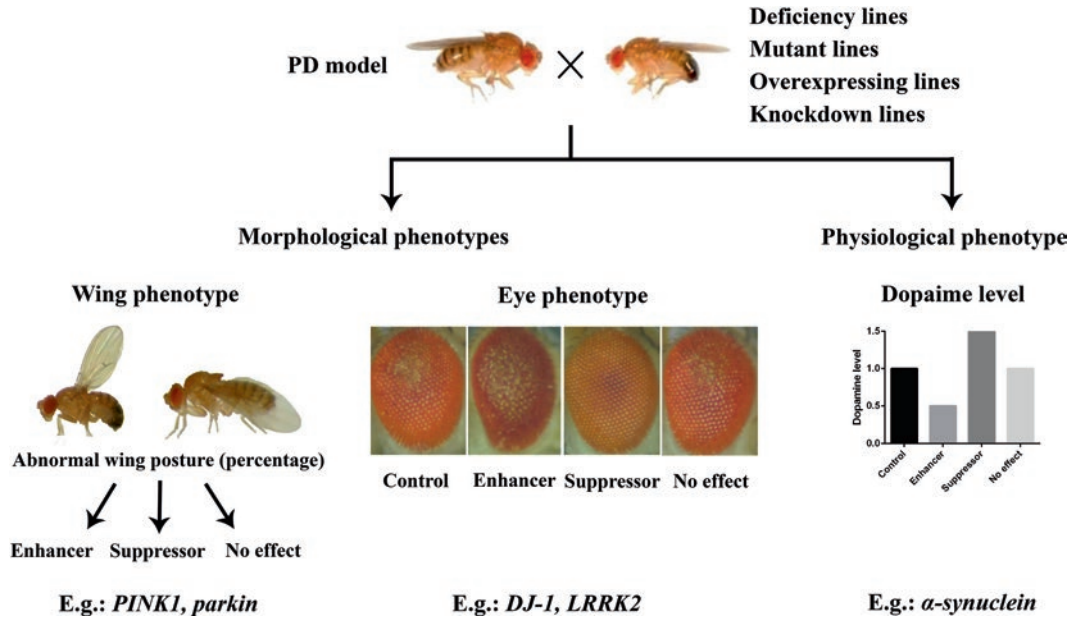

Fig. 4.8 Application of PD-like *Drosophila* model in genetic screening. PINK1, pink (Fernandes and Rao 2011), DJ-1 (Yang et al. 2005), LRRK2 (Venderova et al. 2009), and α-synuclein (Butler et al. 2012)

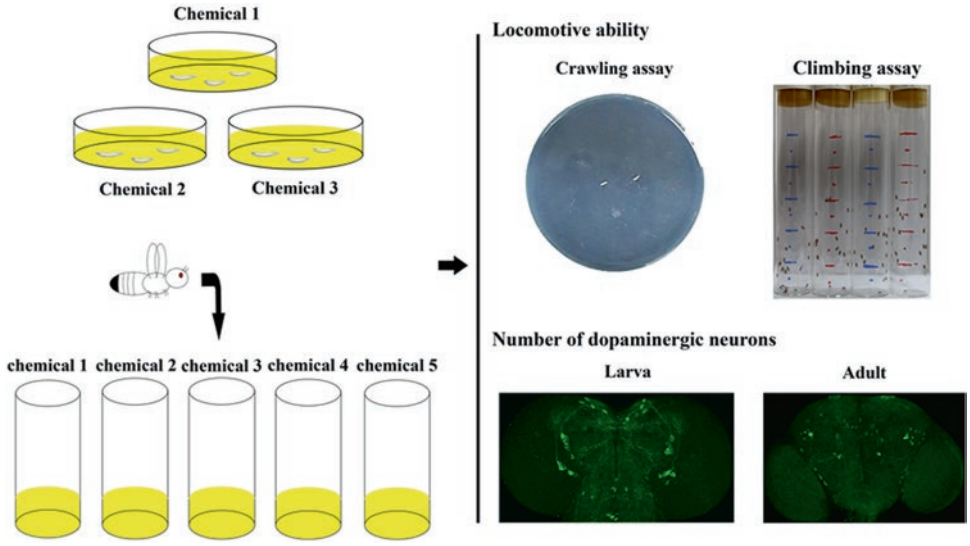

Fig. 4.9 Application of PD-like *Drosophila* model in drug screening

G2019S (Angeles et al. 2016). The other examples of drug screening dVMAT mutant fly were used for screening 1000 known drugs to evaluate the effects of these drugs on locomotor deficits (Lawal et al. 2014). In addition to identifying potential therapeutic compounds, these studies also support the use of *Drosophila* for PD drug discovery.

4.5 Conclusion and Perspective

After Alzheimer disease, PD is the second most common neurodegenerative disease. PD is more commonly associated with motor dysfunction and DA neurodegeneration and is known to show a range of non-motor features. Although many studies demonstrated links of PD to several genetic and environmental factors, mechanism of PD still remains as an interest to investigate. The more PD mechanism is understood, the more advantages in PD therapy and prevention are gained. Currently, it seems to have no potent therapy to cure PD; the application of medicine has just help to control PD symptoms. Therefore, many cellular and animal models of PD have been developed to study PD and discover drug for PD. Among those models, *Drosophila* has been successfully used to mimic PD phenotypes. The *Drosophila* model of PD well displays PD symptoms either motor symptoms, DA neurodegeneration or non-motor symptoms. Owning quite a lot of advantages such as short life span, genetic similarity with PD-related genes, and easiness in maintenance with large populations, *Drosophila* model of PD so far has had a great contribution in PD study. It enables us to further work that may help to understand PD mechanisms, thus identifying new targets for PD treatments.

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