Chapter 11

Understanding Pathophysiology of Sporadic Parkinson's Disease in *Drosophila* Model: Potential Opportunities and Notable Limitations

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting approximately 1% of the population over age 50. PD is widely accepted as a multifactorial disease with both genetic and environmental contributions. Despite extensive research conducted in the area the precise etiological factors responsible remain elusive. In about 95% Parkinsonism is considered to have a sporadic component. There are currently no established curative, preventative, or disease-modifying interventions, stemming from a poor understanding of the molecular mechanisms of pathogenesis. Here lies the importance of animal models. Pharmacological insults cause Parkinsonian like phenotypes in *Drosophila*, thereby modelling sporadic PD. The pesticides paraquat and rotenone induced oxidative damage causing cluster specific DA neuron loss together with motor deficits. Studies in fly PD model have deciphered that signaling pathways such as phosphatidylinositol 3-kinase (PI3K/Akt and target of rapamycin (TOR), c-Jun N-terminal kinase (JNK) have been defective. Further, these studies have demonstrated that fruit fly can be a potential model to screen chemical compounds for their neuroprotective efficacy.

This chapter overviews current knowledge on the pathophysiology of sporadic PD employing *Drosophila* model and discusses the future perspectives. Further we emphasize the importance of performing genome wide screens in fly model, which

may lead to identification of novel pathways involved in PD, which may provide clues to develop therapeutic strategies that help to reduce the burden of PD.

Keywords: Parkinson's disease, *Drosophila*, dopaminergic neurons, neurotoxicants, genome-wide screens

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer disease, affecting approximately 1% of the population over the age of 50. Frequency of PD increases with age, but an expected 4% of people with this disease are detected earlier the age of 50. It is assessed that 7–10 million people worldwide are suffering from PD. About one million Americans are surviving with PD, which is more than the collective number of sufferers diagnosed with muscular dystrophy, Lou Gehrig's disease, and multiple sclerosis. Further, about 60,000 Americans are diagnosed with PD each year and this number does not mirror thousands of unnoticed cases [1]. Studies illustrate that prevalence of PD in men is significantly higher (one and half times more) than in women. In poor and developing nations of Asia and Africa no systematic data are available about the number of sufferers. Painful truth is that in these regions, millions of elderly suffer in silence due to poverty and ignorance.

PD is widely accepted as a multifactorial disease with both genetic and environmental contributions. Clinical signs comprise bradykinesia, resting tremble, muscular rigidity, and postural unsteadiness. Supplementary symptoms are characteristic postural anomalies, dystonic spams, and dementia. PD is progressive and usually has a devious onset in mid to late adult life. Pathogenic characters of typical PD comprise loss of dopaminergic neurons in the *substantia nigra* (SN) and the manifestation of Lewy bodies, intracellular cytoplasmic inclusions, in enduring neurons in various areas of the brain, mainly the SN [2].

Despite intensive research conducted in the field of PD, the etiology of this neurodegenerative disease remains elusive. Although genetic elements and exposure to environmental toxins, such as pesticides, are thought to play a crucial role in disease onset, aging remains the predominant risk factor [3]. In about 95% patients, Parkinsonism is considered to have a sporadic component. Some findings suggest that environmental factors may be more important than genetic factors in familial aggregation of PD. In maximum PD cases the cause is environmental influence, probably toxic, overlaid on a background of slow, sustained neuronal loss due to progressing age [4]. Finding PD in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) drug consumers rejuvenated curiosity in reassessing environmental influences [5]. Another theory of Parkinsonism suggests that genetic predisposition may be transmitted through mitochondrial inheritance.

Current therapeutic strategies for PD mitigate symptoms by the replacement of dopamine, with variable efficacy and considerable side effects. Levodopa (L-dopa), a dopamine precursor, the leading treatment of PD for over 40 years, improves motor impairment by increasing dopamine levels [6]. However, continued use of L-dopa leads to other motor dyskinesias

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that undermine the benefits of treatment. The development of effective treatment for PD is difficult because pathology is affected by several pathways that may act serially or in parallel. However, there are currently no established curative, preventative, or disease-modifying interventions, stemming from a poor understanding of the molecular mechanisms of pathogenesis.

This chapter primarily aims to present an overview of the sporadic PD, disease modeling in *Drosophila* and critically analyze the potential opportunities and the notable limitations associated with fly models. Further, we have also briefly discussed some of the current applications of the model to obtain insights into the underlying molecular mechanism/s related to PD.

2. Animal models of Parkinson's disease

Animal models have been invaluable tools for investigating the underlying mechanisms of the pathogenesis of PD. However, the usefulness of these models is dependent on how precisely they replicate the features of clinical PD. Nonmammalian models are a great cost-effective alternative to rodent and primate-based models, allowing rapid high-throughput screening of novel therapies and investigation of genetic and environmental risk factors. Thus far, the nonmammalian rotenone models have included worm (*Caenorhabditis elegans*), fly (*Drosophila*), zebrafish (*Danio rerio*), and pond snail (*Lymnea stagnalis*). A good model of PD should exhibit pathological and medical characteristics of PD including both dopaminergic and nondopaminergic systems, the central and peripheral nervous systems, also the motor and nonmotor symptoms associated with the disease. Furthermore, the age-reliant inception and progression of pathology should be reflected [7].

Contemporary knowledge on the potential pathogenic and pathophysiological mechanisms of PD derives from innumerable studies conducted, in the past four decades, on experimental models of PD. While animal models, in particular, have provided invaluable information, they also offer the opportunity of trying new therapeutic methods. These model systems have been traditionally grounded on the exposure of neurotoxins able to imitate *many* of the pathological and phenotypic characters of PD in mammals. Conversely in the previous decade, the dawn of the "genetic era" of PD has provided a significant growth in this field with a number of transgenic models for experimentation. It is well recognized that both these classes of animal PD models (genetic and neurotoxin) have their own specificities as well as limitations and employment of one model or the other depends on the specific questions that are being addressed.

Genetic models: Animal models are developed primarily based on identified target genes (i.e., by mutating or knocking out) associated with potential mechanisms known to cause PD in humans (**Table 1**) [8–21]. For example, the autosomal dominant transmission of LRRK2 mutations makes transgenic expression of pathogenic LRRK2 species suitable for modeling disease process in PD. The invertebrate transgenic models producing LRRK2 PD mutants phenotypes range from no change to apparent neuronal loss or deficits in DA systems and

motor behavior [22] that were used to evaluate LRRK2 kinase inhibitors in neuroprotection, revealing the potential value of the invertebrate LRRK2 models in drug screening [23].

Symbol	**Gene locus**	**Gene**	***Drosophila* homolog**	**Inheritance**	**Disorder**	**Status and remarks**
PARK1	4q21-22	SNCA [10]	No homolog	AD	Early-onset Parkinsonism	Confirmed
PARK2	6q25.2-q27	PARK2 encoding Parkin[11]	Parkin	AR	Early onset Parkinsonism	Confirmed
PARK3	2p13	Unknown	–	AD	Classical Parkinsonism	Unconfirmed
PARK4	4q21-q23	SNCA	No homolog	AD	Early-onset Parkinsonism	Erroneous locus (identical to PARK1)
PARK5	4p13	UCHL1	Uch	AD	Classical Parkinsonism	Unconfirmed
PARK6	1p35-p36	PINK1 [12]	Pink1	AR	Early onset Parkinsonism	Confirmed
PARK7	1p36	PARK7 encoding DJ-1[13]	Dj-1α and dj-1β	AR	Early onset Parkinsonism	Confirmed
PARK8	12q12	LRRK2 [14]	Lrrk	AD	Classical Parkinsonism	Confirmed
PARK9	1p36	ATP13A2 [15]	CG32000	AR	Kufor–Rakeb syndrome, a formof juvenile-onset atypical Parkinsonism with dementia, spasticity and supranuclear gaze palsy	Confirmed
PARK10	1p32	Unknown	–	Risk factor	Classical Parkinsonism	Confirmed susceptibility locus
PARK11	2q36-27	Unknown	–	AD	Late	Not

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Symbol	Gene locus	Gene	*Drosophila* homolog	Inheritance	Disorder	Status and remarks
		(maybe GIGYF2)			onset Parkinsonism	independently confirmed
PARK12	Xq21-q25	Unknown	–	Risk factor	Classical Parkinsonism	Confirmed susceptibility locus
PARK13	2p12	HTRA2	HtrA2	AD or risk factor	Classical Parkinsonism	Unconfirmed
PARK14	22q13.1	PLA2G6 [16]	iPLA2-VIA	AR	Early-onset dystonia-Parkinsonism	Confirmed
PARK15	22q12-q13	FBXO7 [17]	No homolog	AR	Early-onset Parkinsonian-pyramidal syndrome	Confirmed
PARK16	1q32	Unknown (maybe RAB7L1)	–	Risk factor	Classical Parkinsonism	Confirmed susceptibility locus
PARK17	16q11.2	VPS35	Vps35	AD	Classical Parkinsonism	Unconfirmed
PARK18	6p21.3	EIF4G1	eIF4G	AD	Late onset Parkinsonism	Unconfirmed
PARK19	1p31.3	DNAJC6 [18]	Auxillin	AR	Juvenile-onset Parkinsonism	Confirmed
PARK20	21q22.11	SYNJ1 [19, 20]	Synj	AR	Early-onset Parkinsonism	Confirmed

AD, autosomal dominant; AR, autosomal recessive (adapted from Marras *et al.* [21]).

Table 1. Monogenetic forms of PD and its fly homolog(s).

Neurotoxic models: Several studies have been performed to model PD-associated neuron loss by neurotoxin intoxication in animals, the most common Parkinsonian neurotoxins being 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP),

rotenone, and paraquat [24, 25], and the common neurotoxic models of PD include that produced by the toxin 6-hydroxydopamine (6-OHDA) commonly used in rats, mice and marmosets, and 1-methyl-4- phenyl-1,2,3,6 tetrahydropyridine (MPTP), used in mice and also in nonhuman primates. Administration of MPTP to animals, such as monkeys, mice, cats, rats, guinea pigs, dogs, sheep and even frogs and goldfish, has been shown to cause Parkinsonian-like motor disturbances [26, 27].

3. Pathophysiology of Parkinson's disease

3.1. Sporadic Parkinson's disease: an overview

A sporadic disease can be explained as a disease occurring randomly in a population with no known cause. In sporadic PD, the cause is considered to be environmental though the genetic influence is also present and hence the pathogenesis of PD is likely to be multifactorial which may involve gene–environment interactions. The discovery of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which reproduces pathological features of idiopathic Parkinsonism by targeting the nigrostriatal system [28] and pesticides (such as rotenone and paraquat), has implicated environmental toxins in the induction of sporadic PD [29, 30]. Both epidemiological and experimental data suggest the potential involvement of specific agents such as neurotoxicants (e.g., pesticides) or neuroprotective compounds (e.g., tobacco products) in the pathogenesis of nigrostriatal degeneration, further supporting a relationship between the environment and PD [28]. Further, the identification of the mutated α-synuclein (SCNA) gene causing familial PD [10] as a risk factor for sporadic disease [31] provides a genetic context for the disease. The finding of α-synuclein as a key component of the Lewy body [32] further links this gene to potential molecular mechanisms of PD.

3.2. Environmental basis of sporadic PD

The study of environmental risk factors for PD is difficult because environmental exposures and gene–environment interactions may occur well before the onset of clinical symptoms since it remains undetected for many years. Moreover, the severe neurodegenerative changes that underlie the symptoms of PD may be the result of synergistic effects of multiple exposures and these effects could have been compounded by increased vulnerability of the aging nigrostriatal system to toxic injury over the years. Epidemiological and case–control studies suggest that rural residence, well water consumption, pesticide use, and certain occupations (farming, mining, and welding) are associated with an increased risk of PD [33–36].

Epidemiological studies have suggested an association with environmental toxins, mainly mitochondrial complex I inhibitors like rotenone [37, 38]. The results are consistent with a dose-dependent effect in agricultural workers and the risk increased with duration of pesticide use [39, 40]. Data also suggest that exposure to specific pesticide such as bipyridyl, organochlorine, and carbamate derivatives could have a causal role in PD [39, 41]. Further, chronic exposure to metals/pesticides is also associated with a younger age at onset of PD among patients with no family history of the disease and that duration of exposure is a factor in the

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magnitude of this effect [42]. For instance, a study in Taiwan, where the herbicide paraquat (PQ) is commonly spurted on rice fields, a robust relationship was testified between paraquat contact and PD menace and the danger was amplified by more than six times in individuals who had been exposed to PQ chronically [43].

3.3. Environment toxins and their mechanisms of action

The accidental discovery of MPTP leading to Parkinsonian syndrome stimulated the search for environmental factors as potential causes of PD. Several epidemiological studies have suggested that environmental toxins are one of the major causes of sporadic PD [44]. Sporadic PD's main cause is the accumulation of alpha-synuclein but by an uncertain causative agent and uneven occurrence point in age of patients. The mechanisms by which the neurotoxins induce PD-like symptoms are briefly described below.

MPTP: MPTP is a metabolite of the drug heroin. It is transported through the blood–brain barrier (BBB) by the plasma membrane dopamine transporter (DAT) and once it crosses the blood–brain barrier, MPTP is metabolically activated to the fully oxidized 1-methyl-4-phenylpyridinium species (MPP+) which is then taken up into dopaminergic neurons via DAT [45, 46]. After MPP+ gains access into dopaminergic neurons, it is accumulated into synaptic vesicles via the vesicular monoamine transporter (VMAT2) [47]. The modulation of MPTP/MPP+ toxicity by DAT and VMAT2, where DAT enhances and VMAT2 protecting against toxicant injury, provides a paradigm linking environmental exposures to nigrostriatal degeneration. The ratio of DAT to VMAT2 indicates the sensitivity of dopaminergic neurons to toxic injury [48].

6-Hydroxy dopamine (6-OHDA)*:* 6-OHDA is the first catecholaminergic neurotoxin that was used to generate animal models of PD. Since this compound cannot cross BBB, it is needed to be injected and inserted systemically to aim dopamine pathways [49]. On injecting into *substantia nigra*, 6-OHDA causes severe loss of dopamine neurons within a day [50]. Inside neurons, 6-OHDA produces reactive oxygen species (ROS) and quinones that inactivate biological macromolecules. Till now, no Lewy body-like inclusion has been described in the 6-OHDA model. Owing to its inability to cross BBB, this model is less popular.

Rotenone (ROT): ROT is used as a broad-spectrum pesticide and belongs to the family of isoflavones naturally found in the roots and stems of several plants. Highly lipophilic, it easily crosses the BBB, and for cellular entry [51], it does not depend on the DAT. Within the cell rotenone mount up in mitochondria and inhibits complex I (where it impedes the transfer of electrons from iron–sulfur (Fe–S) centers to ubiquinone). It is opined that augmented ROS assembly is related with complex I inhibition, which may result in causing oxidative damage to DNA and proteins of neuronal cells. Further, nitric oxide may interact with ROS, particularly superoxide and hydroxyl radicals, resulting in peroxynitrite formation, eventually leading to cellular defects and impairment of dopaminergic neurons [52]. Further, ROT was shown to inhibit proteasome activity and dysfunction in proteasomes has been implicated in the pathogenesis of both genetic and sporadic forms of PD [53, 54].

Paraquat (PQ): PQ is one of the most widely used herbicides in the world. The structural similarity of PQ with 1-methyl-4-phenylpyridinium ion (MPP+) prompted the speculation that PQ might be dopaminergic neurotoxicant which may lead to PD. PQ is suspected to enter the brain by neutral amino acid transporters and subsequently the cells in a sodium-dependent fashion [55]. Once within cells of the CNS, PQ acts as a redox cycling compound at the cytosolic level, which potentially leads to indirect mitochondrial toxicity [56]. Recently, it has also been shown that PQ-induced apoptosis may involve Bak protein, a pro-apoptosis Bcl-2 family member [57].

Maneb (MB): MB, a commonly used fungicide, is an irritant to respiratory tracts and is capable of inducing sensitization by skin contact. Mechanistically, MB seems to cross the BBB. Although knowledge of the mechanisms of this toxin is very limited, MB preferentially inhibits mitochondrial complex III [58]. Further, MB was shown to induce apoptosis through Bak activation, whereas combination of PQ and MB inhibits the Bak-dependent pathway while potentiating apoptosis through Bak protein [59].

Metals: The potential role of metals due to prolonged exposure as risk factors for Parkinson's disease has been evaluated [60]. Chronic occupational exposure to high levels of manganese (Mn) in manganese miners causes accumulation of this metal in the basal ganglia, resulting in tremors, rigidity and psychosis that resemble PD [61]. The metal-induced Parkinsonian syndrome that results from Mn exposure differs significantly from idiopathic PD. The Parkinsonism caused by Mn does not respond to L-DOPA treatment and the primary target of Mn toxicity seems to be the globus pallidus rather than the nigrostriatal system [62]. The potential role of iron and other transition elements has also been studied. The level of ferritin (primary intracellular protein capable of keeping iron bound in a nonreactive status) in the nigral tissue of patients with PD was found to be decreased [63]. Thus, iron accumulation together with decreased binding capability may enhance the risk for iron-mediated toxic reactions in PD by generating the highly toxic hydroxyl radical in the presence of iron and hydrogen peroxide, thus leading to oxidative stress and ultimately neurodegeneration.

4. Molecular pathways in sporadic PD

Though Mendelian genes are responsible only for a small subset of PD patients, it is speculated that the same pathogenetic mechanisms could also play a relevant role in the development of more frequent sporadic PD [64]. With advancement in molecular biotechnological tools and techniques, a number of genes and proteins linked to PD have been identified, which reveal a complex network of molecular pathways involved in its etiology, suggesting that common mechanisms underlie both familial and sporadic forms of PD (**Table 2**) [65–79]. Three predominat pathways that can trigger the neurodegenerative process are as follows: (a) accumulation of aggregated and misfolded proteins, (b) impairment of the ubiquitin protein pathway (UPS) and the autophagy pathway, and (c) mitochondrial dysfunction [64]. Functional studies on the proteins encoded by PD-related genes supports these pathways and it is confirmed by both pathological and biochemical studies performed in patients with sporadic PD with no apparent genetic cause [80–82]. Further, critical cellular protective

http://dx.doi.org/10.5772/63767

pathways, such as autophagy, UPS, and mitochondria dynamics, are shown to lose adeptness with increasing age and there is a progressive build-up of somatic mutations particularly in the mitochondrial DNA during aging process [64]. Recent studies have shown the role for chronic neuroinflammation and microglia activation in PD pathogenesis, suggesting that different molecular/cellular events may contribute to neurodegeneration by activating resident microglial populations in selected brain areas, with potential detrimental effects on vulnerable neuronal populations [83].

Compound treatment	***Drosophila* model**	**Modifies phenotype(s)**	**Pathway/ process**	**References**
Sulforaphane and allyl Disulfide	*parkin*	DA neuron number	Oxidative stress	[65]
	α-synuclein	DA neuron number		[65]
S-Methyl-*L*-cysteine	*α-synuclein*	Locomotor activity		[66]
Polyphenols	*α-synuclein*	Lifespan, Locomotor activity		[67]
	Paraquat and Iron	Locomotor activity		[68]
α-Tocopherol	*DJ-1β*	Lifespan		[69]
	PINK1	Ommatidial degeneration		[70]
SOD	*PINK1*	Ommatidial degeneration		[70]
Melatonin	*DJ-1β*	Lifespan		[69]
	Paraquat	Locomotor activity		[71]
	Rotenone	Locomotor activity, Dopamine neuron number		[71]
Bacopa monieri leaf extract	Paraquat; Rotenone	Oxidative markers; Mitochondrial functions		[72, 73]
Minocycline	DJ-1*α*	DA neuron number, dopamine levels	Oxidative stress/ inflammatory process	[74]
Celestrol	DJ-1*α*	DA neuron number,		[74]

Compound treatment	*Drosophila* model	Modifies phenotype(s)	Pathway/ process	References
		dopamine levels, Locomotor activity and survival rate under oxidative stress conditions		
Rapamycin	Parkin/PINK1	Thoracic indentations, Locomotor activity, DA neuron number, and muscle integrity.	TOR signaling	[75]
Geldanamycin	α-synuclein	DA neuron number	Removal of excess or toxic protein forms	[76, 77]
Zinc Chloride	Parkin	Life span, Locomotor activity, and percentage of adulthood survivors.	Zinc homeostasis	[78]

Modified from Munoz-Soriano and Paricio [79].

Table 2. Therapeutic compounds shown to modify phenotype(s) in the *Drosophila* PD model.

4.1. Genetic basis of sporadic PD

The use of genetically tractable organisms to model gene–environment interactions has become an efficient means of identifying genetic risk factors [84, 85]. Functional characterization of the genes involved in familial PD has shown significant comprehensions into the molecular mechanism(s) responsible to the pathogenesis of PD. Abnormal protein and mitochondrial homeostasis are the crucial factors behind the development of PD, with oxidative stress playing a vital connection between the two events. Genome-wide association studies (GWAS) showed variations in *α-synuclein* and *LRRK2* (well-known familial PD genes), i.e., as important risk causes for the sporadic PD [86]. The elevation of dopamine synthesis in response to a variety of stressors [87] may subject DA neurons to an increased risk for oxidative stress-mediated impairment [88]. Nevertheless, connotation studies of polymorphisms within these genes have not proved the hypothesis [89, 90].

The recent application of high throughput whole genome and exome analysis technologies along with bioinformatics has provided valuable inputs in the identification of novel susceptibility loci involved in apparent sporadic PD. It is predicted that many more variants remained to be discovered despite the success of GWAS in discovering novel genetic variants in PD. In this regard, genome-wide complex trait analysis [91, 92] may prove useful for a more exhaustive screening for PD risk variants [93]. Groundbreaking efforts have begun to establish the relationship between single nucleotide polymorphisms (SNPs) identified by GWAS and gene expression levels to describe their functional meaning. This approach has provided significant insights into various potential novel mechanisms underlying the observed SNP associations with PD etiology.

http://dx.doi.org/10.5772/63767

4.2. Interaction between genetics and environment

The concept that gene–environment interactions affect PD susceptibility was proposed more than a decade ago [94]. Although many studies have described positive associations between genetic polymorphisms and increased risk for PD, only a few human association studies have examined gene–environment interactions. Occupational pesticide exposure as well as high exposure to PQ and MB in carriers of DAT genetic variants was shown to increase the PD risk [36, 95]. Further, SNP in *NOS1* (neuronal nitric oxide synthase 1) and *GSTP1* (glutathione S-transferase pi 1) have been linked to an increased risk for PD among pesticide-exposed individuals [96], although an association between *GSTP1* and pesticide exposure has not been supported by a large cohort study conducted subsequently [97]. However, European studies did not show noteworthy interaction between polymorphisms in 15 genes that impact metabolism of extraneous chemicals or dopamine and exposure to pesticides and metals [97].

Twin studies: Twin studies are particularly useful in distinguishing between the influence of genetics or the environment on the risks of a disease. If genetic factors predominate in etiology of a disease, it is expected that concordance in monozygotic (MZ) twins will be greater than dizygotic (DZ) twins. Using striatal ^{18}F[DOPA] positron emission tomography (PET) scan to detect dopaminergic dysfunction in asymptomatic cotwins of twin pairs with mostly sporadic and late onset PD, Piccini *et al.* [98] found a three-fold higher concordance rate of PD in MZ twins (55%) than in DZ twins (18%), suggesting a significant genetic contribution. Furthermore, when monitored over a period of 7 years, asymptomatic MZ cotwins all showed progressive loss of dopaminergic function and four developed clinical PD, while none of the DZ twin pairs became clinically concordant. Similarly, a recent longitudinal study carried out on Swedish twins with predominantly sporadic PD revealed concordance rates of 11% for MZ and 4% for same-sexed DZ twin pairs, with an overall heritability estimate of 34% [99].

Two-hit PD models: Present genetic PD models failed to reproduce nigrostriatal DA loss, hinting that a single genetic risk factor is not sufficient enough and an environmental factor may be required to initiate the process of neurodegeneration. To understand this paradigm and to decipher the interaction between genes and environment two hit animal models (animals with a genetic defect will be exposed to multiple environmental factors/toxicants to study if this synergy will lead to DA degeneration) will be of potential help.

5. Insights into sporadic PD pathophysiology through *Drosophila*

The fruit fly *Drosophila* has emerged as a suitable model for studying mechanisms of PD-related neurodegeneration in the past decade. Structural architecture and functional pathways involved in dopamine synthesis and degradation are well preserved between *Drosophila* and human. Transgenic flies (neuronal overexpression of *wt* or mutant (A53T or A50P) human alpha-synuclein) showed age-dependent and selective loss of dopaminergic neurons, formation of fibrillary inclusions containing alpha-synuclein, as well as a progressive loss of climbing activity, which could be alleviated by L-DOPA or DA agonists [100]. Mutational analyses of alpha-synuclein in *Drosophila* have permitted an extended evaluation of the protein domains

involved and/or required for toxicity showing, for example, that truncated forms of alpha-synuclein have a central hydrophobic region, between residues 71 and 82, essential for the formation of oligomeric and fibrillary forms of the protein and toxicity. Importance of post-translational modification of alpha-synuclein (phosphorylation on serine 129 and tyrosine 125, on alpha-synuclein oligomerization and toxicity) was demonstrated using the *Drosophila* model. Using fly model it was also shown that early, soluble forms of aggregates of alpha-synuclein are more toxic.

Mutations that induce loss of function or inactivation of the fly homologs of mutations of fly homologs of PINK1, parkin, DJ-1, or LRKK2 lead to selective DA degeneration leading to mobility defects that can be characterized through behavioral assays. *Drosophila* parkin null mutants exhibit decreased life span, mitochondrial abnormalities, and flight muscle deterioration leading to mobility defects and diminished proteasome 26S activity. Overexpression of mutant but not with wild parkin (human gene) in *Drosophila* leads to dopaminergic deterioration and motor defects, signifying a dominant negative effect of the mutated protein in PD pathology. Further, PINK1 mutant flies also share PD characteristics with parkin mutants.

Drosophila models have been important to identify the role of both parkin and PINK1 in the regulation of mitochondrial physiology [101]. Unlike mammals, *Drosophila* expresses two DJ-1 homologs, viz., DJ-1 alpha, restricted to male germline, and DJ-1 beta that, similarly to mammals, is ubiquitously expressed. Different mutations of both genes have been induced. DJ-1beta KO flies showed enhanced susceptibility to cytotoxins, such as paraquat, H_2O_2, and rotenone, further supporting the protective redox function of DJ-1. Similarly, DJ-1beta mutations that cause loss of protein function lead to accumulation of ROS in fly's brain.

5.1. Induction of PD in *Drosophila*

Drosophila were first used to model PD, when Feany and Bender [100] produced transgenic flies that either expressed normal human α-synuclein or one of the mutant forms, A30P and A53T α-synuclein, which have both been linked to familial PD. This discovery revealed the potential of *Drosophila* system for modeling gain and loss-of-function genetic mutations that are associated with PD, thereby allowing the elucidation of the genes molecular functions and the pathways involved.

5.2. Toxin models of *Drosophila* for PD

Several environmental chemicals (neurotoxins) have been employed to recapitulate PD-like symptoms and pathology in *Drosophila* system [102]. *Drosophila* performs motor functions such as walking, climbing, and flying and has a well-developed nervous system which makes *Drosophila* a suitable model for understanding PD. These kinds of complex behavior phenotypes are similar from strain to strain and hence characterizing a toxin induced PD model for this organism becomes easy [100]. Extensively used chemical models with their salient features are briefly described below.

Rotenone (ROT) induced PD model in Drosophila: Inhibition of the mitochondrial respiratory chain by ROT has been widely used to study the role of the mitochondrial respiratory chain

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in apoptosis [103, 104]. The mitochondrial respiratory chain is the major site of ATP production in eukaryotes and it is well recognized that this organelle not only generates ATP, but also plays an important role in apoptosis [105–107]. It is now clear that upon apoptotic stimulation mitochondria can release several proapoptotic regulators, including cytochrome c [108], Smac/Diablo [109, 110], endonuclease G [111], and apoptosis-inducing factor [112] to the cytosol. These proapoptotic regulators will then activate cellular apoptotic programs downstream [105–107]. The release of proapoptotic regulators is further regulated by the translocation of Bcl-2 family proteins [113, 114]. Some of the salient pathophysiological features of the ROT fly model are: (a) being lipophilic, it can easily cross the blood–brain barrier but the final concentration of rotenone in the brain may probably be much lower than the initial because of these barriers and the powerful excretion system of flies. They have a tendency to stay at the bottom of vials and did not appear to coordinate their legs normally [37]. (b) Since neuronal dopaminergic clusters are normally present in each *Drosophila* adult brain hemisphere [115–117], abnormalities are characterized by the disappearance of part or the totality of dopaminergic cell clusters but this effect varies in intensity from one fly to another [37].

Paraquat (PQ) model of PD in Drosophila: Long-term exposure to environmental oxidative stressors, such as the herbicide PQ, has been linked to the development of PD. In view of this, PQ is frequently used in the *Drosophila system* and other animal models to study PD and the degeneration of dopaminergic neurons (DNs). Recently, it has been shown that expression of D_1 like dopaminergic receptor (DAMB receptor) was directly proportional to PQ induced toxicity in CNS of flies [118]. It is notable that a long-term neuronal DA synthesis decreases the DAMB expression and resists the PQ toxicity. Age-related decrement in PQ resistance is also observed with a significant increase in DAMB receptor. This evidence proves that there are more areas to be researched regarding DA related neurodegeneration in *Drosophila*. Some of the salient pathophysiological features of PQ fly model are: (a) flies exhibit rapid onset of movement disorders, including resting tremors, bradykinesis, rotational behaviors and postural instability which resemble Parkinsonian symptoms. Furthermore, the flies frequently freeze while attempting to climb vial walls and would often fall to the bottom of the vial. Males exhibit symptoms 12 hours earlier than females, but both males and females are strongly affected [71]; (b) PQ-dependent dopaminergic neuron loss is totally selective in a time-dependent loss of exposure where after 6 hours of exposure PPL1 and by 12 hours PPM2, PPM3 cluster will be affected whereas PPM1 and PPL2 clusters only get affected after 20–24 hour of exposure [71], and (c) changes in the neuronal cell are also a trait where cell bodies aggregate in a round shape, and fragment and then disappear [71].

6. Application of *Drosophila* model: screening platform for assessment of neuroprotective potential

Drosophila models are a great cost-effective alternative to rodent and primate-based models, allowing rapid high throughput screening of novel therapies. Studies done with *Drosophila* model coexposed to rotenone and melatonin (an antioxidant and free radical scavenger) showed that melatonin improved the movement behavior of rotenone-treated flies, even more

evidently than L-dopa [119]. Quantification of the number of dopaminergic cells after 1 week of rotenone feeding revealed that the presence of melatonin significantly rescued the loss of neurons in all of the clusters [37]. Subsequently, the rotenone model of *Drosophila* has been extensively employed as a screening platform to assess the neuroprotective potential of various molecules and phytoconstituents. Over the last five years, numerous workers have employed the fly rotenone model (both wild type and genetically modified strains) to test potential neuroprotective treatments [72–73, 120, 121]. The majority of these studies used compounds that have multiple therapeutic properties such as antioxidant, anti-inflammatory, and anti-apoptotic properties, which largely yielded positive results such as reductions in ROS and inflammatory mediators, attenuation of TH-positive neuron loss and striatal dopamine loss as well as reversal of motor deficits [122].

6.1. Plant-derived neuroprotective agents in PD

The *Drosophila* model is extensively used due to the flies' rapid generation time, low cost, and amenability for genetic manipulation, and thus serves as an ideal model for identifying promising neuroprotective candidates that can then undergo further validation in mammalian models (**Table 2**) [65–79, 123]. Growing evidence indicate that the herbs used in traditional medicines contain neuroprotective compounds such as resveratol, curcumin or ginsenoside, green tea polyphenols or catechins, triptolide, etc. [124–128]. These compounds may help enhancing antioxidant activity, decrease loss of dopamine, inhibit activation of microglia, reduce the release of pro-inflammatory factors, prevent α-synuclein aggregation and fibrillation. These herbs also protect the dopaminergic neurons against neurotoxins like MTTP, 6-OHDA. Some of the major plant derived molecules suggested as therapeutic agents for PD are as follows.

Resveratol: This is a polyphenolic compound naturally found in grapes. This is able to cross the blood–brain barrier and is water soluble [129]. The numerous pharmacological functions include anti-inflammation, antiapoptosis, antioxidation, anticancer, etc.

Curcumin: In recent years curcumin has shown therapeutic potential for neurodegenerative diseases such as PD. It is a natural polyphenol found in the spice turmeric and is known for several biological and medicinal effects such as anti-inflammatory, antioxidant, anti-proliferative activities, etc. It is demonstrated to help in preventing the aggregation and fibrillation of α-synuclein [130]. Curcumin glucoside, a modified form, prevents the aggregation and enhances the solubility of α-synuclein [131]. Studies have shown that curcumin reduces the LRRK2 kinase activity and decreases the levels of oxidized proteins. Thus curcumin also acts as an inhibitor for LRRK2 kinase activity. Our laboratory has shown stage-specific neuroprotective efficacy of curcumin in *Drosophila* model of idiopathic PD [132].

Ginsenoside: There are two major categories of ginsenosides—protopanaxadiols and protopanaxatriols. In vitro and in vivo studies have shown ginsenosides to exert pharmacological effects against neuroinflammation, cerebral oxidative stress, radical formation, and apoptosis. It plays a neuroprotective role in regulation of synaptic plasticity, neurotransmitter release, and neuroinflammatory responses [126].

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Blueberry extracts: Blueberry contains a large amount of polyphenols and has a greater antioxidant property than most fruits and vegetables. Consumption of blueberry has been reported to slow down the age-related functional and physiological deficits [133–135]. Peng *et al*. [136] were the first to show the anti-aging property of blueberry using *Drosophila* fly model. The study also showed that supplemented blueberry extracts increased the mRNA levels of SOD1, SOD2, and CAT in *Drosophila*. Blueberry extracts can partially reverse the chronic Paraquat exposure. Blueberry extracts in diet of flies could increase the mean life span, decrease Paraquat induced mortality, and partially reverse the locomotor deficiency.

7. Notable limitations

Animal models are absolutely necessary for reproducing physiologic and neurosystems aspects of neurodegenerative disorders. However, animal models are complicated by the differing expression levels and patterns of expression of target genes, with different promoters among other issues for genetic models, and complexities of drug administration, drug distribution, and metabolism for toxin models [79]. Rodent models have faced limitations due to lack of strong construct (i.e., genotype or intervention) and face validity (i.e., phenotype), as well as species and strain limitations. 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 [137, 138], but they have been useful to support the concept that alterations in mitochondrial biology are essential for the development of PD [139]. However, animal models allow studying a cellular process in the context of a whole organism and are thus more reliable.

Research on PD using cell cultures has many advantages in which they allow rapid screening for disease pathogenesis and drug candidates. Cellular models can be easily used for molecular, biochemical, and pharmacological approaches, but they can lead to misinterpretation and artifacts. *Vice versa* limitations include that the survival of neurons is dependent upon the culture conditions and the cells do not develop their natural neuronal networks. In most cases, neurons are deprived of the physiological afferent and efferent connections [140].

While there are many advantages of the fly PD model, the most common disadvantage is that the important pathogenetic factors which are vertebrate-specific may be ignored in invertebrate models. The differences between mammals and invertebrates represent potential drawbacks in modeling brain diseases such as PD [141].

8. Potential opportunities

Drosophila melanogaster was the first major complex organism to have its genome sequenced [142] and after the human genome was sequenced the homology between the two genomes greatly strengthened to understand human biology and the disease processes as a model [143]. More importantly, 75% of human disease-related loci have a *Drosophila* orthologue [144]. Fly

model are less costly and time consuming to use when compared to mammals due to their rapid reproduction time and short lifespan [143, 145, 146]. In addition, flies are capable of performing complex motor behaviors such as walking, climbing, and flying and their brain is complex enough to make these behaviors relevant to humans [101, 147, 148].

Some of the unique features of the *Drosophila* model which have been identified are: (a) *Drosophila* models are instrumental in exploring the mechanisms of neurodegeneration, with several PD-related mutations eliciting related phenotypes including sensitivity to energy supply and vesicular deformities. These are leading to the identification of plausible cellular mechanisms, which may be specific to (dopaminergic) neurons and synapses rather than general cellular phenotypes. (b) Fly models show noncell autonomous signaling within the nervous system, offering the opportunity to develop our understanding of the way pathogenic signaling propagates, resembling Braak's scheme of spreading pathology in PD, (c) fly models link physiological deficits to changes in synaptic structure, and (d) the strong neuronal phenotypes observed in the fly models permit relevant *in vivo* drug testing [149]. Another key feature making *Drosophila* an attractive model is the range of genetic tools available to manipulate them and the ease of introducing human genes into the fly enables it to recapitulate the symptoms and progression of human disease in flies [150]. Two approaches employed are: the *reverse genetic approach* wherein a gene is tested for its potential functional role by using the GAL4/*UAS*-system and the *forward genetic approach* (function of a gene) for identification of genes based on phenotype, which is useful to understand diseases whose genetic basis is yet to determined [141]. The genomics era has played a crucial role in directing both the functional biology and the *in vitro*/*in vivo* modeling of neurodegenerative diseases in fly model.

9. Future perspectives

Drosophila has been used to model several aspects of neurodegenerative diseases, including aggregation toxicity of misfolding disease related proteins [151–156]. Ninety-five percent of the Parkinson's disease patients suffer from sporadic form. In those sporadic cases, no indication allows a decided inference about the underlying causes as well as the pathogenic mechanism involved [101]. The limitations of human genetics make it necessary to use model system to analyze affected genes and pathways knowledge of which is essential to develop therapeutic targets. During last three decades, genetically pliable fruit fly *Drosophila* has been a great model system to study human neurodegenerative disorders including PD human genetic screens, and pathological studies have been able to provide limited mechanistic insights into the molecular processes that determine disease susceptibility or age at onset of disease [157]. Genetic analysis has identified causative mutations for autosomal-dominant and recessive forms of familial PD. Functional studies of these genes have provided great insights into potential pathogenic mechanisms of inherited forms of PD; however it is unclear how these may relate to the more common sporadic forms of PD.

Identification of PD risk locus SREBF1 through GWAS (genome-wide association studies) analysis and substantiating its biological function as a regulator of mitophagy [158] remarka-

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bly emphasize the importance and potential to decipher the risk loci for idiopathic PD through genome-wide screens in animal models. However, no systematic genome-wide functional screens are performed in sporadic PD models. Here lies the importance and necessity to perform genome-wide screen to identify the risk locus for idiopathic PD. Comprehensive efforts in this direction will provide novel insights into the molecular mechanisms behind the dopaminergic neurodegeneration and also figure out genetic basis for sporadic PD. Here lies the potential relevance and advantage of fly genetics and available technologies such as UAS-Gal4, fly deletion lines, and RNAi lines, which can be of great help to figure out novel players, pathways, and mechanistic interactions among neurodegenerative disorders. Hence, it is worth placing future endeavors in this direction.

10. Conclusion

In this chapter, we have provided an overview of current knowledge on the pathophysiology of sporadic PD employing *Drosophila* system. We also presented the future perspectives on the subject matter and emphasize the utmost importance for the need to generate comprehensive data employing genome-wide association studies in this model that may lead to identification of newer pathways. We also discussed the importance and necessity to reexamine the strategies/methods of screens to assess the potential of neuroprotective compounds/molecules employing *late life stages* that may provide us better answers on successful utilization of therapeutic compounds in late onset neurodegenerative disorders such as PD.

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