CELL MODEL OF DJ-1-ASSOCIATED

PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive loss of motor function resulting from dopaminergic neuronal death in the *substantia nigra pars compacta* leading to subsequent decreased striatal dopamine levels. The majority of PD cases are diagnosed as sporadic in nature, however 10% - 15% of patients show a positive family history of the disease. While many genes have been found to be implicated in the familial form of PD, early-onset autosomal recessive PD has been associated with mutations in PARK7, a gene which codes for the protein DJ-1. While there are many proposed roles of DJ-1 across numerous systems, the function of DJ-1 in relation to the development and progression of PD remains largely unclear. A first step towards determining this function is the creation of biologically relevant cell models of PD. The goal of this work was to design a representative cell model of DJ-1-associated PD in order to further study DJ-1 with the intention of elucidating its relevant function in relation of PD pathogenesis.

Quyen Hoang, Ph.D., Chair

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LIST OF ABBREVIATIONS

AD	Autosomal dominant
AR	Autosomal recessive
C-terminal	Carboxyl-terminus
CO_2	Carbon dioxide
COR	C-terminal of ROC
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EOPD	Early-onset Parkinson's disease
FBS	Fetal bovine serum
IBR	In-between-RING
kDa	Kilodalton
LRR	Leucine-rich repeat
LRRK2	Leucine-rich repeat serine/threonine-protein kinase 2
MAPKKK	Mitogen-activated protein kinase kinase kinase
MAOI	Monoamine oxidase inhibitor
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance image
N-terminal	Amino-terminus
NAC	Non-amyloid-β component
NADH	Nicotinamide adenine dinucleotide
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction

PD	Parkinson’s disease
PEI	Polyethylenimine
pI	Isoelectric point
PINK1	PTEN-induced putative kinase 1
PTEN	Phosphatase and tensin homolog
REM	Rapid eye movement
RING	Really interesting new gene
RNA	Ribonucleic acid
ROC	Ras of complex protein
ROS	Reactive oxygen species
SV40	Simian vacuolating virus 40
UBL	Ubiquitin-like
UCH-L1	Ubiquitin carboxy-terminal hydrolyase 1
UPS	Ubiquitin-proteasome system
WD40	40 amino acid motif ending in a tryptophan-aspartic repeat
wt	Wild type

INTRODUCTION

1.1 PARKINSON'S DISEASE

Parkinson's disease is a progressive degenerative neurological disorder that is estimated to affect 1 in 100 people age 65 and older[1,2] and 3 in 100 people age 75 and older.[3] The overall incidence rate is roughly 13.4 per 100,000 after adjusting for gender and age. There is a higher prevalence among men, 19.0 per 100,000, than among women, 9.9 per 100,000, and demonstrates a similar incidence rate across most ethnicities.[4]

1.2 HISTORY OF PARKINSON'S DISEASE

Parkinson's disease was first characterized at length by Dr. James Parkinson in 1817 in his publication, *An Essay on the Shaking Palsy.* In this manuscript, he described the progression, outcome and some of, what are known as, the cardinal symptoms of PD, distinguishing them from other confounding diseases.[5] Many questions Parkinson expressed in the early 1800s are still much the same even 200 years later. Until the late twentieth century, genetic predispositions were thought to play an insignificant role in the development of PD. The prevailing belief was that environmental factors, not genetic, led to the onset of PD. This idea was reaffirmed by a post-encephalitic outbreak of PD in the early 1900s. However, a number of papers dating back to as early as 1900 have noted that individuals with PD often have an affected relative and even provided documented evidence of the Mendelian inheritance of PD.[6,7] Interestingly though, two papers were published showing a low rate of concordance in both monozygotic and dizygotic twins, again suggesting an absence of genetic influence on PD.[8,9] To further bolster this argument, in the early 1980s, a strain of synthetic heroin was determined to

be the cause of an outbreak of aggressive, rapid-onset parkinsonism cases. The toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was found to be the responsible contaminate.[10] MPTP preferentially enters dopaminergic neurons resulting in the inhibition of complex I of the electron transport chain. This finding elucidated mitochondrial dysfunction as a contributing factor to the development of PD. This also proved to be a landmark finding that provided a mechanism by which models of PD could be studied in animals and *in vitro*. The idea that PD may have a genetic component was not revisited until the 1990s when Marder et al. published a study demonstrating the increased risk of PD among first degree relatives.[11] Genetic susceptibility was finally confirmed by the first pathological mutation conclusively shown to cause PD.[12]

1.3 EARLY-ONSET PARKINSON'S DISEASE

While the average age of onset is estimated to be around 60 years of age, this is not the only cohort to be affected by PD. Early-onset PD (EOPD) is classified as having an onset before the age of 50; however there have been diagnoses of PD in individuals as young as 18, perhaps even younger.[13] The overall incidence rate of PD in individuals aged 30-39 is roughly 0.5 per 100,000 and 1.6 per 100,000 for those aged 40-49.[4] Though these numbers may seem insignificant, EOPD makes up nearly 10% of all PD cases.[14] Furthermore, studies in the past few years have suggested that both the incidence of EOPD[15] and PD,[16] in general, are on the rise. This could be attributed, in part, to the considerably high rate of pathogenic mutations found in two genes known to cause EOPD in comparison to genes responsible for classic or late-onset PD.[17,18] Even the third

most commonly mutated gene, albeit a distant third, primarily known to cause late-onset PD, has been shown to also be a risk factor for EOPD.[19]

1.4 CLINICAL MANIFESTATIONS OF PARKINSON'S DISEASE

While PD is often associated with tremors, some symptoms of Parkinson's disease are hard for even specialists to detect. Parkinson's symptoms can be different for every patient; each case is unique and the pace at which the disease progresses can vary on an individual basis. The general public is usually most familiar with the motor symptoms of PD, as these are the signs of the disease that manifest most clearly from the outside. These symptoms, known as the cardinal symptoms of Parkinson's disease include resting tremor, slowness of movement and rigidity. Many people also experience balance problems (postural instability) and other physical symptoms, such as gait problems and reduced facial expression.[20] These symptoms often appear gradually and with increasing severity over time. Typically, they begin on one side of the body and with time migrate to the other side.[21]

There is increasing recognition of both the presence and effects of non-motor symptoms of PD, sometimes called "dopamine-non-responsive" symptoms. These symptoms are seen more commonly in non-classical PD cases; however even classic PD is not completely exempt. Symptoms can include: cognitive impairment, mood disorders, problems sleeping such as REM sleep disorder, low blood pressure when standing, speech and swallowing problems as well as loss of smell.[22] Considering the list of symptoms and current treatment options, it is evident that no one medication or treatment will work to minimize all symptoms or work in all people. That being said,

there are a number of fruitful treatment options for Parkinson's disease, the most common being dopamine replacement therapy, aimed at encouraging the brain to synthesize dopamine with the replacement compound as a precursor. Dopamine agonists as well as MAOIs are also prescribed to treat Parkinson's. Deep brain stimulation as well as a number of experimental trials are also possible treatment options. At the moment, there is no objective test or biomarker available for reliable diagnosis of PD so misdiagnosis is possible; however most research suggests there are at least one million people in the United States with PD and more than 10 million worldwide.[23] That being said, most certain diagnosis of PD can be made post-mortem.

1.5 PATHOLOGICAL MANIFESTATIONS OF PARKINSON'S DISEASE

Lewy bodies are insoluble, eosinophilic cytoplasmic inclusions typically found in the substantia nigra pars compacta, but are not localized only to this area.[24] These protein aggregates are considered one the neuropathological hallmarks of PD and is used as a post-mortem criterion for diagnosis of PD.[25] Lewy bodies consist primarily of α-synuclein but it is not known what molecular events lead to their formation.[26] Large deposits of ubiquitinated protein suggest that their aggregation may be due, in part, to proteasomal impairment or even inhibition.[27,28] In support of this idea, studies have shown a decreased level of proteasomal activity in the substantia nigra of PD patients.[29,30] However, because unmodified α-synuclein is typically degraded by the proteasome independent of ubiquitination, it is possible that this modified α-synuclein may overwhelm the proteolytic pathway and instead cause abnormal ubiquitination.[31]

Another pathological hallmark of PD is the selective death of dopaminergic neurons in the substantia nigra. Interestingly, the effects of this can be seen in the brain, post-mortem, without staining or imaging techniques as tyrosine, dopamine's precursor, is a dark, melanin color; two neuromelanin steaks across the substantia nigra indicate a healthy individual, where light or non-existent streaks are symptomatic of PD.[32,33]

1.6 GENETICS AND HERITABILITY OF PARKINSON'S DISEASE

Over 20 years after the first description of a causative mutation for PD, it has become evident just how complex this disease is. Roughly 30 distinct chromosomal regions have been related to PD, less than a third of these regions, however, contain genes with mutations that definitively cause monogenic PD.[34] Even then, these mutations only account for 3-5% of disease occurrences suggesting that PD may be caused by an association of multiple factors. A non-exhaustive list could include: unreported pathways, multiple genes, risk factors/ susceptibility alleles, gene-environment interactions or environmental exposure.

Suspected PD-causing genes are given the nomenclature of *PARK* and are numbered in chronological order of their initial report, i.e. *PARK1*, *PARK2*, *PARK3*, etc. This list is not comprehensive and not without its shortcomings. First, this list is compromised of both verified loci and loci for which the findings could not be replicated, as is the case with UCH-L1. Furthermore, the causative gene for each loci has not yet been determined, such as *PARK3*. That being said, another shortcoming is that not all *PARK* genes are causative; a number of *PARK* genes are, instead, risk factors for PD: *PARK10, PARK12, PARK16*.

All currently documented monogenic forms of PD are autosomal, recessive or dominant, meaning that they are found on autosomally-linked chromosomes rather than the sex-linked. Autosomal-dominant disorders require only one mutation to display the phenotype while autosomal recessive require both alleles to carry the mutation. Autosomal dominant mutations are able to overcome the normal phenotype in one of three ways: haploinsufficiency – meaning that a single copy of the normal allele is not able to compensate to provide appropriate function of the protein; the dominant negative effect – where the mutant phenotype is nonfunctioning causing it to inhibit the endogenous function of the normal allele; or the opposite effect, a gain-of-function mutation – when the mutation causes the protein to gain a new, unendodgenous function.[34] While Mendelian inheritance patterns provide a streamlined theory of disease manifestation, *in vivo*, diseases rarely follow these patterns for a number of reasons.[35] These patterns often become convoluted by factors such as reduced penetrance – failing to express the trait even though one may carry the allele,[36] and variable expression – differences in the way individuals may express the associated disease phenotype.[37] Differences seen in disease phenotype may also be a result of molecular aspects such as the location of mutation (functional domains, binding pockets, etc.) which could lead to a number of consequences in function.

Most pathogenic PD mutations have been discovered through gene mapping or candidate gene approaches. Gene mapping is a method used to identify location of genes in relation to molecular markers and can be used with no prior pathological hypothesis as it takes the entire genome into account.[12,36] Gene mapping techniques

include linkage analysis and genome-wide association studies. Candidate gene approach, instead, is based on associations between genetic variants within genes of interest associated with specific disease phenotypes.[38] With advances in technology and increasing accessibility of the genome, the latter approach has become outdated; however, there may still be instances, such as polymorphisms with low allelic frequency, in which candidate gene approach would be more appropriate.[39] Nonetheless, these studies, have helped to identify over 20 *PARK* genes as well as a myriad of other genes thought to play a role in the development and progression of PD (*Table 1*). Select genes have been explained in detail and are the subject of the next section.

Table 1. Genes implicated in Parkinson's disease

Locus	Chromosome	Protein (Gene)	Inheritance Pattern	Phenotype	Reference
PARK1	4q21-q22	α-synuclein (SNCA)	AD	Early onset, Lewy Body Dementia	[40]
PARK2	6q25.2-q27	parkin (PRKN)	AR	Early onset, slow progression	[41]
PARK3	2p13		AD	Classic PD	[36]
PARK4	4q21-q23	α-synuclein (SNCA)	AD	Early onset, Lewy Body Dementia	[42]
PARK5	4p13	UCH-L1	AD	Classic PD	[43]
PARK6	1p35-p36	PINK1	AR	Early onset, slow progression	[44]
PARK7	1p36	DJ-1	AR	Early onset, slow progression	[45]
PARK8	12p11.2-q13.1	LRRK2	AD	Classic PD	[46]
PARK9	1p36	ATPase type 13A2 (ATP13A2)	AR	Classic PD, dementia	[47]
PARK10	1p32		Risk factor	Classic PD	[48]
PARK11	2q36-q37	GRB10 interacting GYF protein 2 (GIGYF2)	AD	Late onset PD	[49]
PARK12	Xq21-q25		Risk factor	Classic PD	[48,50-52]
PARK13	2p12	HtrA serine peptidase 2 (OMI/HTRA2)	AD	Classic PD	[53]
PARK14	22q13.1	A2 phospholipase (PLA2G6)	AR	Early onset PD with dystonia	[54]
PARK15	22q12-q13	F-box protein 7 (FBXO7)	AR	Early onset with dementia	[55]
PARK16	1q32		Risk factor	Classic PD	[56]
PARK17	16q11.2	VPS35	AD	Classic PD	[57]
PARK18	3q27.1	Eukaryotic translation initiation factor (eIF4G1)	AD	Classic PD	[58]
PARK19	1p31.3	DNAJC6	AR	Early onset PD	[59]
PARK20	21q22.11	SYNJ1	AR	Early onset PD	[60]
PARK21	3q22		AD	Classic PD	[61]
PARK22	7p11.2	CHCHD2	AD	Classic PD	[56]
PARK23	15q22.2	VPS13C	AR	Early onset PD	[62]

Table 1 cont. Genes implicated in Parkinson's disease

Locus	Chromosome	Protein (Gene)	Inheritance Pattern	Phenotype	Reference
	5q23.1-q23.3	Synphilin-1 (SNCAIP)	AD	Classic PD	[63]
	2q22-q23	Nuclear receptor subfamily 4, group A, member 2 (NR4A2)	AR	Classic PD	[64]
	19q13.32	Apolipoprotein E [ε2, ε4 alleles] (APOE)	Risk factor	Earlier onset of PD, increased risk of dementia	[65-67]
	1q22	Glucosylceramidase Beta (GBA)	Risk factor	Classic PD	[68]
	17q21.31	Microtubule Associated Protein Tau (MAPT)	Risk factor	Classic PD	[69,70]
	20p13-p12.3	Transmembrane Protein 230 (TMEM230)	AD	Classic PD with Lewy Body Dementia	[71]
	15q26.1	Polymerase DNA gamma (POLG)	AR	Early onset	[72]
	5q31.2	mortalin (HSPA9)	AD	Early onset	[73]
	3q27.1	Presenilin-associated rhomboid like protein (PARL)		Early onset	[74]
	8p22	N-acetyltransferase 2 (NAT2)	Risk factor	Classic PD	[75]
	4p16.3	Cyclin G-associated kinase (GAK)	Risk factor	Classic PD	[76]
	6p21.32	HLA-DRA	Risk factor	Classic PD	[77]

1.6.1 α-SYNUCELIN

The first gene linked to familial PD was mapped to chromosome 4q21-q23 through a multigenerational study of an Italian-American family, the Contursi kindred, which examined more than 400 members over 5 generations and identified 60 affected individuals who displayed autosomal dominant disease inheritance with early onset progression.[12] An A53T missense mutation was found in the gene coding for α-synuclein in these affected individuals.[40] Later, a second mutation would be found in a German family, A30P,[78] and a third in a Spanish family, E46K.[79] To date, there are roughly 30 chromosomal regions associated with PD in α-synuclein; however less than 10 are known to be monogenic.[34] α-synuclein is a highly conserved, 140 amino acid protein that is particularly abundant in the presynaptic terminals of neurons where it associates with membrane and vesicular structures.[80-82] It is part of a larger family of synucleins, which include β-synuclein and γ-synuclein.[83] α-synuclein contains multiple domains: an N-terminal amphipathic region consisting of six imperfect repeats and is proposed to have a mitochondrial targeting sequence,[84] a hydrophobic region where the non-amyloid-β component (NAC) domain resides, and an acidic C-terminal. Depending on the environment, α-synuclein can vary structurally; α-synuclein is intrinsically unstructured, or is a natively unfolded protein, but can take on conformations as monomeric or oligomeric species or can form aggregated protein known as amyloids.[85] The molecular function of α-synuclein remains abstruse. Normal function is thought to include roles such as dopamine transport regulation and dopamine release, fibrilization of MAPT and neuroprotective ability in non-dopaminergic cells/neurons. [86-89]

Pathologically, α-synuclein is a key identifier of PD and other synucleinopathies as it is the major structural component of Lewy bodies.[90] Mutations in α-synuclein are thought to lead to PD through a gain-of-function mechanism which causes increased expression of the protein and self-aggregation, ultimately leading to Lewy body formation; compared with wild-type α-synuclein, both the A30P and A53T mutants exhibit an increased propensity to self-aggregate and form oligomeric species.[91] It is unclear how α-synuclein produces its downstream cytotoxic effects. Many arguments have been made suggesting a direct interaction between α-synuclein and the ubiquitin-proteasome system (UPS).[92-94] However, alternative pathways have also been considered including the autophagy, or lysosomal degredation, pathway.[95] Some have suggested that the intermediates of oligomerization, protofibrils, may be the pathogenic cytotoxic culprit rather than the fibrils themselves. This proposal came from the observation that A30P and A53T mutants are capable of inducing the oligomerization but not fibrilization of α-synuclein.[96] Furthermore, soluble protofibrils are observable in human brain tissue of PD patients, indicating oligomeric species may be physiologically relevant.[97] However, it has been demonstrated that β-amyloid promotes formation of α-synuclein fibril-inclusions in bigenic mice overexpressing α-synuclein and mutant amyloid precursor protein leading to a more severe pathological phenotype.[98] Additionally, increases in α-synuclein fibrilizaiton has been associated with proteasomal inhibition causing formation of insoluble fibril-inclusions in both primary neuronal cultures *in vitro*[99] and *in vivo*.[100] Delineation of the precise mechanism in which α-synuclein contributes to

increased cellular toxicity in dopaminergic neurons will be key to understanding its role in PD.

1.6.2 PARKIN

In its initial discovery, a large region on chromosome 6q25.2-q27 was linked to a rare form of autosomal recessive juvenile onset PD in multiple consanguineous Japanese families.[101] Shortly thereafter, a homozygous deletion was found in a microsatellite marker of an individual affected with parkinsonism symptoms, the adjacent gene was appropriately named, parkin.[41] Mutations in the parkin gene are fairly common in familial PD. Mutations are found in 50% of early-onset, recessive, familial cases and 10% of all early-onset cases.[17] A large variety of pathogenic mutations have been described from small deletions to hundreds of kilobases, repeats as well as missense mutations.[102,103] Through a genome-wide scan of families with early-onset PD, there was significant linkage to only parkin, indicating a critical role for the gene in the development/progression of early-onset PD.[52] The parkin gene encodes a protein 465 amino acids in length. It contains an N-terminal ubiquitin-like (UBL) domain and a C-terminal RING domain with two RING fingers and an in-between-RING (IBR) domain. Parkin can function as an E3 ubiquitin protein ligase through its RING finger motifs,[104,105] and can interact with E2s as well as substrate proteins through its RING domain. Parkin has been shown to interact with E2 enzymes, UbcH7 and UbcH8,[58] and with UBC6 and UBC7 which are endoplasmic reticulum-associated E2s.[106] Pathogenic mutations are considered loss-of-function mutations and weaken interactions of parkin through its RING domain, by either hindering its E3 ubiquitin protein ligase activity or by blocking

interactions with E2s and substrates. These mutations are thought to prompt improper targeting for proteasomal degredation causing neurotoxic accumulation. A proteomic analysis of parkin knockout mice showed decreased concentrations of proteins involved in mitochondrial oxidative phosphorylation, such as NADH-ubiquinone oxidoreductase, pyruvate dehydrogenase E1α1 and cytochrome c oxidase, as well as peroxiredoxins 1,2,6 which are thought to function as antioxidants offer protection from oxidative stress.[107] These reductions were further associated with decreases in the repiratory capacity of mitochondria and increases in age-related oxidative insult suggesting a role for parkin in the matenince of mitochondrial function. Parkin knockouts in other models have also yielded interesting results; *Drosophila* parkin knockouts showed reduced lifespan, motor deficits (due to apoptotic muscle degeneration) and male sterility. These symptoms are important indicators of mitochondrial dysfunction that are often accompanied by increased oxidative stress.[108] How parkin mitigates oxidative sress and stimulates the survival of dopimergic neurons is still unknown. Overexpression of parkin in cell culture not only demonstrates a resistance to agents known to induce mitochondria-targeted apoptosis but also shows a localization to the outermitochondrial membrane.[109] Parkin overexpression has been shown to protect against proteasome-inhibition-induced toxicity as well as protection against increased expression of mutant α-synuclein.[110] Furthermore, in *Drosophila* models, overexpression of parkin has been shown to rescue dopaminergic neurons from α-synuclein-positive inclusions, indicating that parkin may function to relieve or deter aggregations of

mutant α-synuclein.[111] Further investigation into the interacting partners of parkin may present alternative mechanisms of protection against cytotoxicity in relation to PD.

1.6.3 PINK1

Through a genome-wide homozygosity screening of a Sicilian family, a 12.5 cM region on chromosome 1p35-p36 was found as a commonality across 4 family members with early-onset PD.[112] This region was later found to have positive linkage from unrelated families and was thereby confirmed as a relevant, pathological gene.[113] Further study revealed a handful of mutations in the PINK1 gene concentrated mostly across its serine/threonine protein kinase domain.[114] PINK1 is 581 amino acids in length, contains an N-terminal mitochondrial targeting sequence, the aforementioned serine/threonine protein kinase domain and a C-terminal autoregulatory domain. Although mutations of PINK1 are most commonly found in exon 7 (serine/threonine protein kinase domain), they affect all 8 exons with similar frequencies.[115] Mutations in PINK1 are thought to contribute to PD through loss-of-function demonstrating the importance of the enzymatic activity of PINK1 in the pathogenesis of PD. PINK1 has been shown to use its kinase activity to phosphorylate mitochondrial proteins in response to oxidative stress in order to prevent mitochondrial dysfunction.[44] Furthermore, it has been suggested that PINK1 and parkin interact through a common pathway, known as the PINK1/parkin pathway,[116] as a form of quality control for the mitochondria: screening and eradicating damaged mitochondria from the mitochondrial network. PINK1 localizes to the mitochondria, where it is stabilized by a lower membrane potential and then recruits

cytosolic parkin. This recruitment causes parkin to become enzymatically active and stimulates removal of the mitochondria through mitophagy.[117]

1.6.4 LRRK2

Genome-wide linkage analysis of a Japanese family with autosomal dominant PD showed positive linkage to chromosome 12p11.23-q13.11.[46] LRRK2 mutations are the most common, known cause of late-onset autosomal dominant PD.[118] While mutations are common, the reported pathological findings are inconsistent, ranging from Lewy body pathology to substantia nigaral degeneration without Lewy bodies, both with and without neurofibrillary tangles.[119] LRRK2 encodes a cytoplasmic protein that is 2527 amino acids in length. It contains an N-terminal leucine-rich repeat (LRR) domain, a ROC (Ras of complex protein) domain, COR (C-terminal of ROC) domain, a mitogen-activated protein kinase kinase kinase (MAPKKK) domain and a C-terminal WD40 repeat domain.[120] There are upwards of 50 reported missense and nonsense mutations,[121] of those mutations, at least 16 are deemed to be pathogenic in nature.[122] Interestingly, these pathogenic mutations are concentrated to 10 exons, encoding the C-terminal kinase region.[123] The mechanism by which LRRK2 dysfunction leads to PD is unknown. Because LRRK2 is a large protein with many domains capable of their own unique functions and possibility for an infinite number of protein-protein interactions, there is much work to be done to tease apart the pathological interactions or functions.

1.6.5 UCH-L1

Candidate gene-screening identified previously unreported mutation, heterozygous I93M, in the gene encoding ubiquitin carboxyl-terminal hydrolase L1 in an affected

sibling pair. Interestingly though, the parent transmitting the mutation was found to be asymptomatic.[43] This may suggest that the I93M mutation is nonpathogenic or that the mutation may cause PD through incomplete penetrance. Another mutation, S18Y, was found through a case-control study.[124] However, following studies failed to replicate these findings with consistency. UCH-L1 is 212 amino acids in length and is located on chromosome4p13. It is found in high concentrations in neurons and is part of a deubiquitinating family of enzymes that hydrolyze ubiquitin chains to free monomeric ubiquitin.[125] It has been suggested that UCH-L1 may also function as a dimerization-dependent ubiquitin protein ligase which acts to maintain ubiquitin homeostasis by binding and stabilizing ubiquitin monomers in neurons. [125,126] UCH-L1 has also been shown to localize to Lewy bodies and can promote accumulation of α-synuclein in cell culture.[127,128] It is thought that mutation of UCH-L1 hinders its ubiquitin function causing progression of PD; however it is unclear from current literature if this is pathologically relevant or if the reported mutations were the result of a coincidental polymorphism.[129,130]

1.6.6 DJ-1

In 2001, homozygosity mapping of a family with multi-consanguinity affected with EOPD demonstrated significant evidence for linkage on chromosome 1p36. [131] Shortly thereafter, multiple monogenic mutations were found in studies of Italian and Dutch families.[45] Following these findings, novel mutations including deletions, splice site alterations and missense mutations were documented in a number of other families with EOPD.[132] Today, roughly 20 pathogenic mutations have been reported in DJ-1 in

relation to EOPD cases (*Table 2*). However, mutations to DJ-1 comprise only a very small percentage of EOPD cases, roughly 1-2%.[133,134]

DJ-1 encodes for a highly conserved protein 189 amino acids in length belonging to the DJ-1/ThiJ/PfpI family and is currently only known to have a single functional domain (*Figure 1*).[135] DJ-1 is ubiquitously expressed in both the brain and periphery.[136,137] It is found in neurons[45,138] and microglia,[139] but is expressed in the highest amount in astrocytes.[140-145] DJ-1 was originally discovered 20 years ago as an oncogene that transformed cells in conjunction with H-Ras.[146] Studies have confirmed that DJ-1 expression is greatly increased in multiple types of cancer[147,148] but more importantly, this oncogenic role may relate to regulation of the phosphatase and tensin homolog (PTEN) tumor suppressor,[149] the substrate of which, is necessary for pathways related to PD.[150] Later investigations into the role of DJ-1 yielded a variety of activities including a protective protein present in rat sperm,[151] a modulating protein for androgen-receptor mediated transcription[152] and part of the regulatory component of RNA-binding protein complexes.[153]

The physiological function of DJ-1 is unknown. Most evidence suggests that DJ-1 may function as a redox sensor or an antioxidant protein.[154-157] In cultured cells exposed to oxidative stressors, DJ-1 exhibits an acidic shift in isoelectric point (pI). This shift can be explained by the oxidation of cysteine residues which can be converted to a cysteine-sulfinic acid ($C\text{-}SO_2H$).[138,158] Furthermore, DJ-1 is able to self-oxidize in order to eliminate hydrogen peroxide (H_2O_2) signifying a potential role as a direct-scavenger of reactive oxygen species.[159] Overexpression of DJ-1 protects against oxidative insult in cell culture

while knockdown of DJ-1 increases susceptibility to oxidative stress.[159] However, cellular stress does not only modify the state and function of DJ-1, it also causes a change in its location. Various studies have demonstrated that oxidative stress causes DJ-1 to localize to the outer membrane of the mitochondria. Here, it is believed to mitigate mitochondria-dependent cell death.[138,160,161] It has been documented that DJ-1 localizes to the mitochondria in response to oxidative stress and then moves to the nucleus, all within a specific time frame.[138,161,162] Interestingly, DJ-1 lacks any known mitochondrial or nuclear targeting sequences. In separate studies, DJ-1 has been shown to interact with parkin,[163,164] PINK1[165-167] and α-synuclein[154,156,168] under oxidative stress conditions which could suggest that linkage in a larger functional pathway. In addition, through knockdown and overexpression studies, DJ-1 has been shown to prevent cell death by regulating endoplasmic reticulum stress as well as proteasome inhibition.[169] These findings suggest that DJ-1 may play a significant role in protecting against cell death.

1.6.6.1 CLINICAL OVERVIEW

Symptoms of EOPD can vary significantly, making them more difficult to diagnose. Less than half of patients experience the resting tremor characteristically associated with PD. Patients with EOPD also have a slower disease progression and are more responsive to treatment, specifically L-dopa.[170] EOPD typically presents with dystonia – muscle contractions which lead to abnormal presentations of limbs or posture. According to clinical studies, EOPD patients have a higher rate of dyskinesia – impairment or difficult with voluntary movement, and a worse quality of life when compared to classic PD patients.[170-172] Furthermore, there is a higher incidence of non-motor symptoms in

EOPD but a lower rate of psychological and cognitive symptoms.[170] Symptoms alone are not enough to confidently diagnose EOPD; however imaging studies may offer better insight. An Italian MRI study found that the use of three markers – unilateral R2 of the substantia nigra, fractional anisotrophy of the right substantia nigra and mean diffusivity in the caudate nucleus or putamen – were able to accurately distinguish between EOPD patients and controls.[173]

1.6.6.2 PATHOLOGY

Unlike classic PD, EOPD caused by DJ-1 is not associated with the presence of Lewy bodies. However, it has been suggested that DJ-1 may be able to associate into filaments because of its homodimer confirmation. Crystal structures revealed that DJ-1 dimers were stacked linearly and formed into protofilaments facilitated by inorganic phosphate, and then bundled into filamentous structures.[174] It is known that the solubility of DJ-1 is altered resulting in the formation of aggregates and insoluble DJ-1 aggregates have been found in brains of PD patients.[142,175-177] However this study only observed DJ-1 aggregation *in vitro*, warranting further study, or replication, *in vivo*. Subsequently, it remains unclear how this filamentous confirmation contributes to the development or progression of PD.

1.6.6.3 GENETICS AND SEQUENCE VARIANTS

Pathogenic mutations are thought to cause PD through a loss-of-function mechanism, the most notable of these being the L166P mutant. This mutation was shown to cause destabilization of the entire protein and promotes unfolding of the C-terminus.[45] Unfolding of the C-terminal region leads to a loss of dimerization and is thereby an

easier target for degradation by the proteasome.[177-179] The L166P mutant has also been shown to diminish the neuroprotective ability of DJ-1 in cell culture, most likely as a direct consequence of its lack of stability.[159] Monogenic mutations in DJ-1 have also shown to affect their ability to interact with other proteins suggesting that interactions with such proteins are part of normal function and integral to maintaining their role in the cell.[165] Such mutations and their effects are listed in *Table 2*.

Table 2. Pathogenic mutations of DJ-1

Mutation	Inheritance Pattern	Effect	Reference
L10P	homozygous	Protein instability, aggregation	[180,181]
M26I	homozygous	Protein instability	[182]
A39S	heterozygous	Reduction in basal levels of PINK1	[165]
E64D	homozygous	unknown	[183]
R98Q	heterozygous	Polymorphism	[184 185]
G115T	homozygous	unknown	[165]
A104T	heterozygous	Increased conformational stability	[186 187]
D149A	heterozygous	unknown	[182]
E163K	homozygous	Impaired activity	[188 189]
L166P	homozygous	Protein instability	[45]
A179T	heterozygous	unknown	[190]
g.168_185dup	homozygous	unknown	[188]
Ex1-5dup	homozygous	unknown	[190]
P158del	homozygous	Protein instability, aggregation	[181,190]
c.56delC	heterozygous	Frameshift, protein truncation	[186]
g.168_185del	heterozygous and homozygous	Polymorphism	[186]
14-kb del	homozygous	Loss of functional protein	[45]
Ex5-7del	heterozygous	Altered sequence	[184]
IVS5+2-12del	heterozygous	Altered sequence	[184]
IVS6-1 G-C	heterozygous	Altered sequence	[186]

1.6.6.4 STRUCTURAL BIOLOGY

The crystal structure of DJ-1 revealed a flavodoxin-like fold similar to that of PH1704, a bacterial protease, and Hsp31, a stress-induced homodimeric protein and homolog of human DJ-1, from *Escherichia coli* and yeast.[191-193] These structures also revealed that DJ-1 exists as a dimer with eight α-helicies and 11 β-strands arranged into a helix-turn-helix sandwich,[177,178,194] typical of the ThiJ/PfpI superfamily (*Figure 2*).

Much debate has been centered on whether the active site of DJ-1 is considered a diad or triad; proponents of the triad point out the similarities to catalytic triad active sites (C-H-D/E) with C106, H126 and E18 filling those roles. Catalytic cysteine residues are conserved across the ThiJ/PfpI superfamily.[191] However this orientation is not as favorable as the orientation of normal cysteine proteases and does not seem to be appropriate for functional proton transfer.[193] This suggests that DJ-1 may instead have a catalytic diad consisting of C106 and H126 only[195] thereby distinguishing itself from other structural homologs.

Cysteine residues are known to act as redox-sensitive indicators in a variety of proteins.[196] DJ-1 contains three cysteine residues C46, C53 and C106. Of these three, it has been shown that C106 is the most sensitive to oxidative stress.[197] C106 is easily oxidized to cysteine-sulfinate ($C106\text{-}SO_2^-$) under very mild conditions and even unintentionally. [138] Structural analysis revealed that this moiety is stabilized by three hydrogen bonds to adjacent residues, including one to the protonated COOH side chain of E18, an argument in favor of the necessity of E18 in catalytic function. [138]

Mutational analysis of the cysteine residues – C46A, C53A and C106A – revealed diminished oxidation and decreased ability to mitigate cell death in C106A mutants only.[138] Furthermore, it has been shown that C106A binds with Bcl-X_L, a mitochondrial protein located on the outer mitochondrial membrane, far less than *wt* DJ-1 suggesting that the ability for C106 to be oxidized may be integral for the localization of DJ-1 to the mitochondria.[198]

Figure 1. Domains of DJ-1. Designed based on the following studies. [135,192,199]

findings. [191,193,199-201]

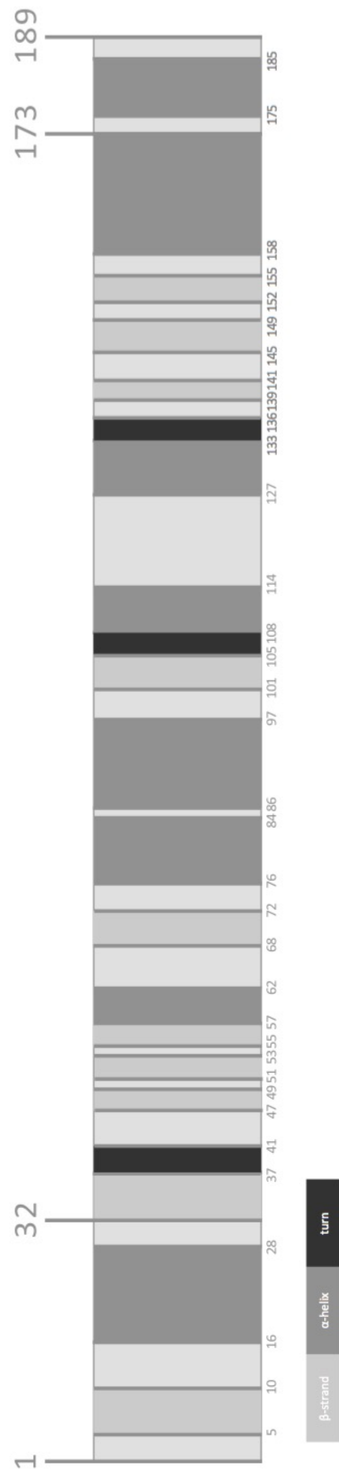

Figure 2. Map of the secondary structure of DJ-1. Designed based on the following findings. [191,193,199-201]

1.7 MODELS OF PARKINSON'S DISEASE

The first models of PD were developed after Parkinson-like systems were seen in a number of patients who had been exposed to a contaminated strain of synthetic heroin, as discussed earlier. Today models of Parkinson's disease often aim to replicate PD through genetic mutations, knock-downs, knock-outs, overexpression and toxin-induced stressors, such pesticides and various complex I inhibitors, in both *in vivo* and *in vitro* approaches.[202] In this particular model, we used neuroblastoma cell lines infected with our constructs, discussed in full detail later, in order to overexpress the *wt* and mutant proteins, respectively.

1.7.1 NEUROBLASTOMA CELL LINES

Neuroblastomas are malignancies that form in nerve cells. Samples taken from cancerous cells are often used for cell culture studies due to their ability to divide indefinitely. This ability is due to the fact that they produce telomerase, where normal, non-cancerous, cells do not. Telomerase is an enzyme that adds to telomere repeat sequences at the ends of chromosomes to prevent degradation of telomeres and subsequent replicative senescence, where cells cease to divide. Neuroblastoma lines, SH-SY5Y and M17, were chosen for use in this study. While cells *in vitro* can be differentiated using substances like retinoic acid to induce further cellular specialization, cell lines in this study were not differentiated. It has been shown that undifferentiated SH-SY5Y and M17, opposed to differentiated, cell lines are more representative of catecholaminergic/dopaminergic neurons which would be most appropriate for the study of Parkinson's disease.[203-205]

1.7.1.1 SH-SY5Y

SH-SY5Y cells were originally derived from a metastasized neuroblastoma via a bone biopsy. The parental cell line, SK-N-SH was subcloned three times, first to SH-SY, then SH-SY5 and finally into SH-SY5Y. SH-SY5Y cells are genetically female and contain neuroblastoma morphology and are positive for enzymes tyrosine hydroxylase and dopamine-β-hydroxylase both of which are characteristic components of dopaminergic neurons.[206] SH-SY5Y cells contain a low level of tyrosine hydroxylase, the rate-limiting enzyme of the catecholamine synthesis pathway, responsible for creating dopamine's precursor from tyrosine, dopamine can then be further converted to epinephrine by dopamine-β-hydroxylase.[207]

1.7.1.2 M17

M17 cells were cloned from SK-N-Be(2) neuroblastoma cells collected from a metastasized site. While the SH-SY5Y cells are genetically female, M17 cells are genetically male and were derived from the bone marrow of a 2-year-old boy. M17 cells also contain neuronal marker enzymes and enzymes involved in the catecholamine synthesis pathway.[208,209]

1.7.2 OXIDATIVE STRESS

Various toxins have been used to induce oxidative insult that leads to cell death modeling the selective death of dopaminergic neurons due to oxidative stress. Popular models include MPTP, rotenone, 6-hydroxydopamine paraquat and menadione.[202,210,211] These compounds lead to mitochondrial dysfunction in various ways leading to reactive oxygen species (ROS) production and subsequent cell death.

1.7.2.1 MENADIONE

Vitamin K3, or menadione, is a mild oxidative stressor. It was chosen as it allows easier control over concentrations, most consistent stressor used by the lab.[211] It is thought that menadione generates ROS through redox cycling, but the exact mechanism is still unclear.[212]

1.8 LENTIVIRAL TRANSFECTION AND TRANSDUCTION

Lentiviruses are a form of retrovirus utilized in cell culture because of their ability to integrate genetic material into host genomes regardless of what stage of the cell cycle the cell is in.[213] Furthermore, by integrating into the host genome, lentivirus are able to create stable cell lines opposed to transient cell lines made by most traditional methods of transfection.[214]

1.8.1 PACKAGING VECTORS

Packaging vectors are required to supply the proper encapsulation and machinery of the virus. Different generations of lentiviral expression systems require different packaging vectors. This system required three vectors; pRRE, pRSV-REV and pVSV-G. The first, pRRE, contains three components: gag which codes for the structural viral proteins, pol which is responsible for retrovirus-specific enzymes and RRE, a binding site for the REV protein. The second, pRSV-REV, contains REV which facilitates the export of RNA from the nucleus. The third, pVSV-G, contains the vesicular stomatitis G protein, the envelope plasmid, which enables budding from the packaging cell, utilizing the packaging cell membrane to create the membrane of the virus.[215] All three packaging vectors were a gift from the lab Dr. Clark Wells.

1.8.2 PACKAGING CELL

The packaging cells we used, 293T *dtx*, were also a gift from the Wells lab. Originally derived from a human kidney cell line, 293T cells are known for their high transfection efficiency due to its expression of a mutant SV40 large T antigen.[216] These cells are most commonly used for expression and production of retroviruses.[217] Through the addition of the three packaging vectors, 293T cells serve as the host for the viral components; the membranes of which become the membrane of the virus through budding.

METHODS AND MATERIALS

2.1 CONSTRUCTS AND VECTORS

The *wt* human DJ-1 construct was a gift to the lab from the late Dr. Anthony Fink of the University of California Santa Cruz (*Figure 3*). This construct was mutated using Q5 Site-Directed Mutagenesis to create the DJ_{C106A} mutant. Presence of the mutation was verified via sequencing (*Figure 4*).

Lentiviral acceptor vectors, mCherry and 3x FLAG, were obtained from the Wells Lab at Indiana University School of Medicine. Empty vectors with no acceptor components of mCherry and 3x FLAG were also obtained to serve as controls (*Figures 5 and 6*).

2.2 CLONING

ASCI/PACI restriction enzyme sites were cloned into *wt* and mutant DJ-1 constructs using PCR. Following PCR, all constructs and vectors were double restriction enzyme digested overnight. The restriction enzyme digest products were run on a DNA gel and the appropriate bands were extracted. The extracted gel products were then purified and ligated for a minimum of 2 hours. The ligation products were transformed into DH5α cells, spread on plates and incubated at 37°C overnight. Colonies were selected and subjected to colony PCR to confirm presence of the insert. Colonies containing the insert were then selected and cultured overnight. Plasmid purification was performed the next day and samples were sent out for sequencing as secondary verification (*Figures 7-10*).

Nucleic acid sequence:

ATGGCTTCCAAAAGAGCTCTGGTCATCCTGGCTAAAGGAGCAGAGGAAATGGAGACGGTCATCCCTGTA
GATGTCATGAGGCGAGCTGGGATTAAGGTCACCGTTGCAGGCCTGGCTGGAAAAGACCCAGTACAGTGT
AGCCGTGATGTGGTCATTTGTCCTGATGCCAGCCTTGAAGATGCAAAAAAAGAGGGACCATATGATGTG
GTGGTTCTACCAGGAGGTAATCTGGGCGCACAGAATTTATCTGAGTCTGCTGCTGTGAAGGAGATACTG
AAGGAGCAGGAAAACCGGAAGGGCCTGATAGCCGCCATCTGTGCAGGTCCTACTGCTCTGTTGGCTCAT
GAAATAGGCTGCGGAAGTAAAGTTACAACACACCCTCTTGCTAAAGACAAAATGATGAATGGAGGTCAT
TACACCTACTCTGAGAATCGTGTGGAAAAAGACGGCCTGATTCTTACAAGCCGGGGGCCTGGGACCAGC
TTCGAGTTTGCGCTTGCAATTGTTGAAGCCCTGAATGGCAAGGAGGTGGCGGCTCAAGTGAAGGCTCCA
CTTGTTCTTAAAGACTGA

Amino acid sequence:

MASKRALVILAKGAEEMETVIPVDVMRRAGIKVTVAGLAGKDPVQCSRDVVICPDASLEDAKKEGPYDVVVL
PGGNLGAQNLSESAAVKEILKEQENRKGLIAAICAGPTALLAHEIGCGSKVTTHPLAKDKMMNGGHYTYSENR
VEKDGLILTSRGPGTSFEFALAIVEALNGKEVAAQVKAPLVLKD

Figure 3. Nucleic acid and amino acid sequences of *wt* DJ-1.

Nucleic acid sequence:

ATGGCTTCCAAAAGAGCTCTGGTCATCCTGGCTAAAGGAGCAGAGGAAATGGAGACGGTCATCCCTGTA
GATGTCATGAGGCGAGCTGGGATTAAGGTCACCGTTGCAGGCCTGGCTGGAAAAGACCCAGTACAGTGT
AGCCGTGATGTGGTCATTTGTCCTGATGCCAGCCTTGAAGATGCAAAAAAAGAGGGACCATATGATGTG
GTGGTTCTACCAGGAGGTAATCTGGGCGCACAGAATTTATCTGAGTCTGCTGCTGTGAAGGAGATACTG
AAGGAGCAGGAAAACCGGAAGGGCCTGATAGCCGCCATC**GCT**GCAGGTCCTACTGCTCTGTTGGCTCAT
GAAATAGGCTGCGGAAGTAAAGTTACAACACACCCTCTTGCTAAAGACAAAATGATGAATGGAGGTCAT
TACACCTACTCTGAGAATCGTGTGGAAAAAGACGGCCTGATTCTTACAAGCCGGGGGCCTGGGACCAGC
TTCGAGTTTGCGCTTGCAATTGTTGAAGCCCTGAATGGCAAGGAGGTGGCGGCTCAAGTGAAGGCTCCA
CTTGTTCTTAAAGACTGA

Amino acid sequence:

MASKRALVILAKGAEEMETVIPVDVMRRAGIKVTVAGLAGKDPVQCSRDVVICPDASLEDAKKEGPYDVVVL
PGGNLGAQNLSESAAVKEILKEQENRKGLIAAI**A**AGPTALLAHEIGCGSKVTTHPLAKDKMMNGGHYTYSEN
RVEKDGLILTSRGPGTSFEFALAIVEALNGKEVAAQVKAPLVLKD

Figure 4. Nucleic acid and amino acid sequences of DJ-1 C106A mutant. Mutation denoted in bold with underline.

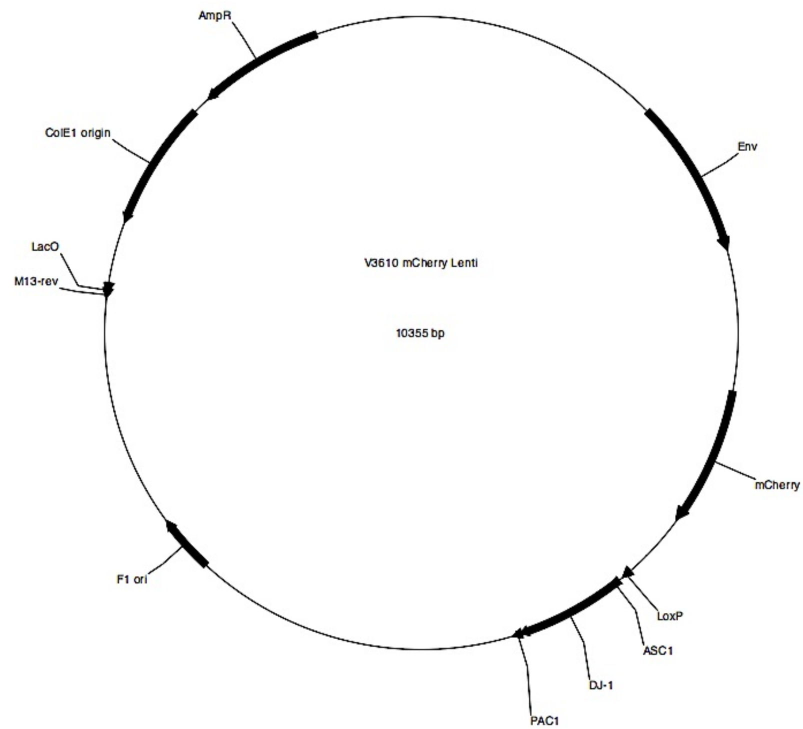

Figure 5. Vector map of empty mCherry lentivirus.

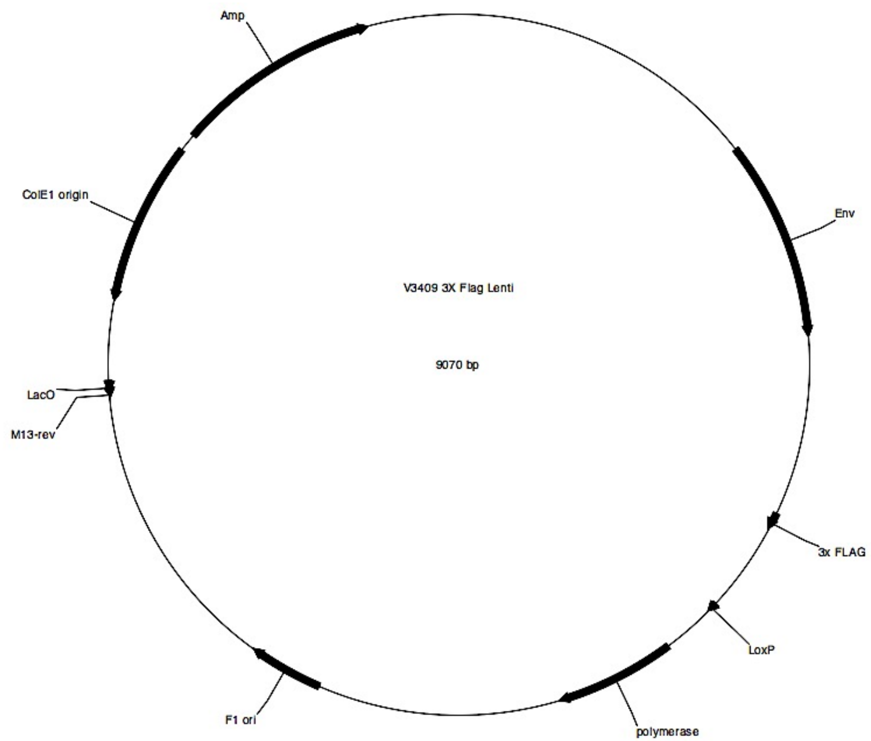

Figure 6. Vector map of empty 3x FLAG lentivirus.

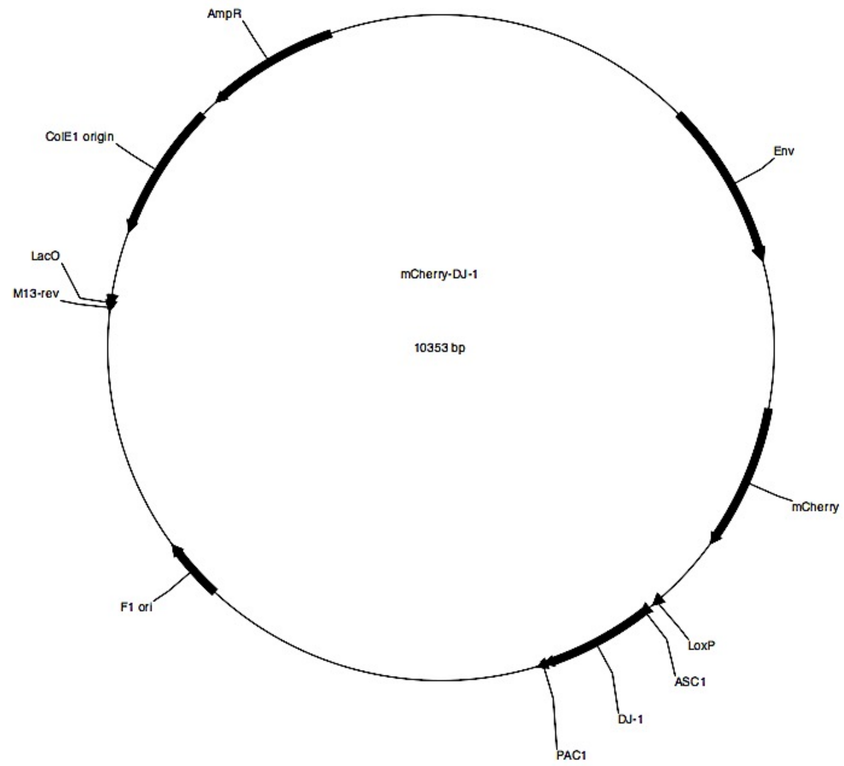

Figure 7. Vector map of mCherry-DJ-1.

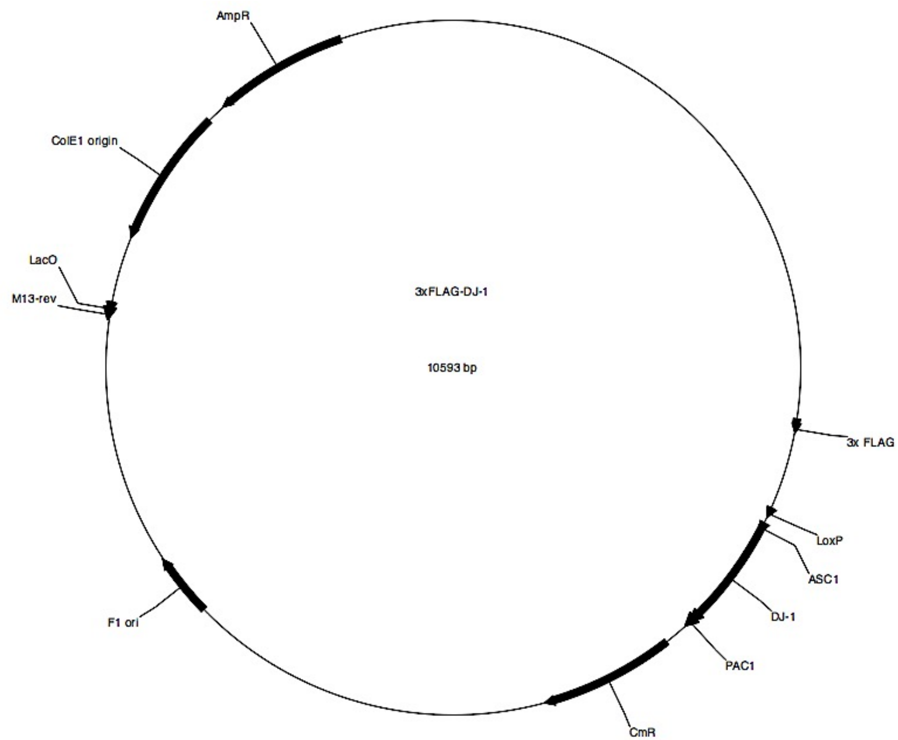

Figure 8. Vector map of 3x FLAG-DJ-1.

Figure 9. Vector map of mCherry-DJ-1 C106A.

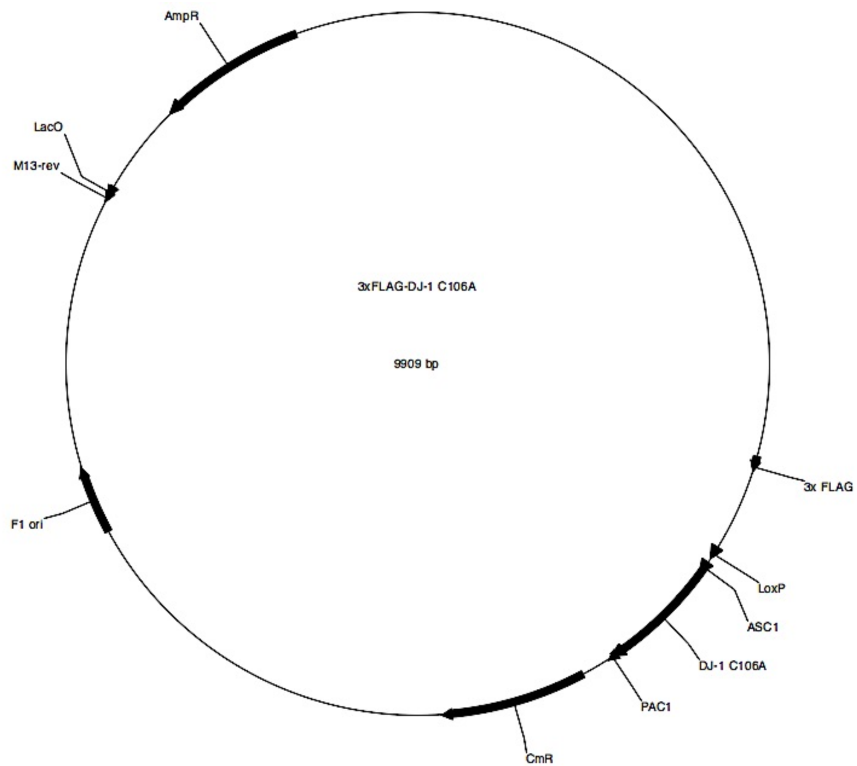

Figure 10. Vector map of 3x FLAG-DJ-1 C106A.

2.3 THAWING AND PLATING OF CELLS

Cyrotubes containing empty cell lines were thawed in a 37°C water bath briefly and removed when only a small piece of ice remained. The outside of the vial was sprayed with 70% ethanol and were added drop-wise immediately to a plate containing 10 mL complete media. The plate was then transferred to the 5% CO_2/95% air humidified incubator at 37°C.

2.4 MAINTENANCE OF CELL LINE

Two different neuroblastoma cell lines (M17 and SH-SY5Y) were infected with our constructs, 293T dtx cells were used to create virus. All cell lines were grown in 10 cm polystyrene plates and kept in a 5% CO_2/95% air humidified incubator at 37°C. Both neuroblastoma cell lines were maintained in OptiMEM medium supplemented with 10% fetal bovine serum (FBS), while 293T dtx cells were maintained in DMEM medium with 10% FBS. Medium was changed every other day. Cells were passaged once they reached 75-85% confluency. Following passage, media was changed after 4 hours.

2.5 PASSAGE OF CELLS

Old media was removed and replaced with 5 mL PBS to wash the cells. PBS was then removed as well and 1 mL 0.05% trypsin-EDTA was added drop-wise to each plate. Plates were then placed into the 5% CO_2/95% air humidified incubator at 37°C for no more than 3 minutes. Complete media (OptiMEM + 10% FBS) was then added to inactivate the trypsin. Cells were then counted and plated at the appropriate density in plates containing 10 mL complete media.

2.6 TRANSFECTION OF VIRUS

293T dtx cells were grown to 65-70% confluence in 10 mm plates. In styrene tubes, 1 mL DMEM, 6 µg pVSV-G, 5 µg pRSV-Rev, 10 µg pRRE and 20 µg lentiviral DNA construct were combined. The tube was vortexed while simultaneously adding 50 µg PEI drop-wise. The tube was left to sit for 5 minutes, then the mixture was added drop-wise to the 293T dtx plates and incubated for 4-8 hours in the 5% CO2/95% air humidified incubator at 37°C. The transfection media was then replaced with complete medium and left to incubate for 48 hours in the 5% CO_2/95% air humidified incubator at 37°C. The viral media was then collected and transferred to a 15 mL conical vial. Polybrene was added and the mixture was centrifuged at 400xg for 2 minutes.

2.7 INFECTION OF NEUROBLASTOMA CELLS

Cells were grown to 80% confluence, media was removed and then washed with PBS. Collected viral media was combined with complete medium in a 1:3 ratio of viral to complete media for SH-SY5Y cells and a 1:2 ratio for M17 cells. Cells were then incubated for 4-8 hours in the 5% CO_2/95% air humidified incubator at 37°C. The viral media was then replaced with complete medium and cells were placed back into the 5% CO2/95% air humidified incubator at 37°C.

2.8 PREPARATION OF WHOLE CELL LYSATES

Protein expression after oxidative stress was analyzed in M17 and SH-SY5Y cell lines via Western blot. Cells were plated at a density of 1.0 x 10^6 cells in 100mm polystyrene plates. Cells were collected by washing once with ice-cold 1x PBS and then detached

with 0.05% trypsin-EDTA. Trypsin was neutralized by complete media, then the cells were pipetted into 15 mL conical tubes, centrifuged for 2 minutes at 1100 rpm. Supernatant was removed and cells were resuspended in 1x PBS. Cells were immediately put on ice and then sonicated.

2.9 DETERMINATION OF TOTAL PROTEIN CONCENTRATION

Bradford assay was used to determine protein concentration in M17 and SH-SY5Y cells. A 1:1000 ratio was used (i.e. 1 uL sample to 1mL 20% Bradford assay dye). Protein concentrations were measured in NanoDrop using a previously generated protein standard curve through the use of known concentrations of a protein standard.

2.10 PREPARING AND RUNNING PROTEIN GEL

Twenty-five mg of total protein was loaded for both cell lines and across all constructs. The volume needed for the protein load was determined using the protein concentration obtained via Bradford assay. The appropriate amount of 2x loading dye was added to each sample and were boiled for 2-3 min to denature the proteins. The samples were loaded on a 12.5% polyacrylamide gel and run at 20V for 10 minutes then 120V for 1 hour and 30 minutes.

2.11 DETECTION OF PROTEIN VIA WESTERN BLOT

Proteins from the gel were transferred to PVDF membrane overnight at 30V at 4°C. The next day, membranes were washed with PBS-T (450 mL ddH2O, 50 mL 10x PBS and 2.5 mL Tween 20) and then blocked for 2h at room temperature in blocking buffer to prevent non-specific antibody binding. After blocking, primary antibodies were added

directly to the blocking buffer and incubated for 3 hours on an orbital shaker at room temperature. Membranes were then washed 3 times with PBS-T for 10 minutes at a time. Blocking buffer plus secondary antibodies were added and left to incubate for 45 minutes at room temperature on an orbital shaker. Membranes were washed again, 3 times for 10 minutes at a time in PBS-T. Membranes were rinsed with ddH_2O and left for 10 minutes at room temperature on the orbital shaker. Protein bands were then visualized using a Licor scanner.

RESULTS

3.1 OVEREXPRESSION OF CONSTRUCTS IN NEUROBLASTOMA CELLS

In order to evaluate if both cell lines had been infected and were overexpressing, they were first visualized using a fluorescent microscopy. Vectors containing the mCherry tag would fluoresce. Full transfection could be visualized 48 hours post-transduction (*Figure 11*). Verification of all constructs was done via Western blot. Anti-β-actin and anti-DJ-1 primary antibodies were used to confirm expression of DJ-1. Anti-β-actin (42 kDa) was blotted as control and is red (*Figures 12-15*). Anti-DJ-1 showed both endogenous and tagged DJ-1 (*Figures 12 and 14*) and is green in these particular blots. DJ-1 is endogenously expressed in both SH-SY5Y and M17 cells. Endogenous DJ-1 (20kDa) is the lower band across all lanes while tagged DJ-1 is the upper band in lanes 5 and 6; the shift in size is due to the extra 28.8 kDa from the mCherry tag or 2.9 kDa from the 3x FLAG tag. Further validation was obtained using anti-β-actin and anti-FLAG (*Figures 13 and 15*). Anti-FLAG primary antibody was used to independently visualize 3x FLAG-DJ-1, lane 6, as the 3x FLAG constructs are not fluorescent. Empty 3x FLAG is too small to appear on Western blots, explaining a lack of banding in these lanes. In the anti-β-actin and anti-FLAG blots, the upper green bands are β-actin at ~42 kDa while the lower band in lane 6 is 3x FLAG-DJ-1 (~23 kDa).

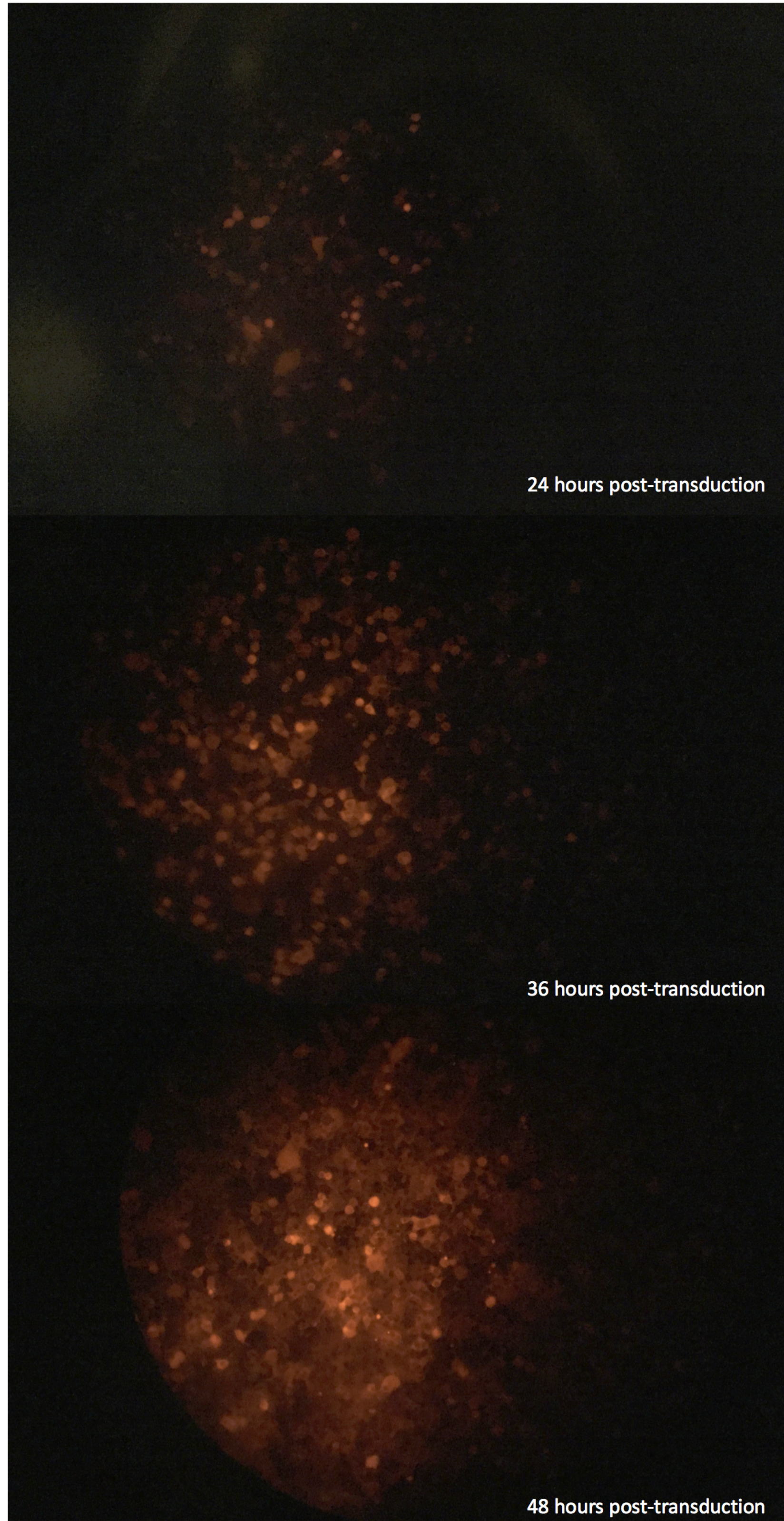

Figure 11. mCherry fluorescence.

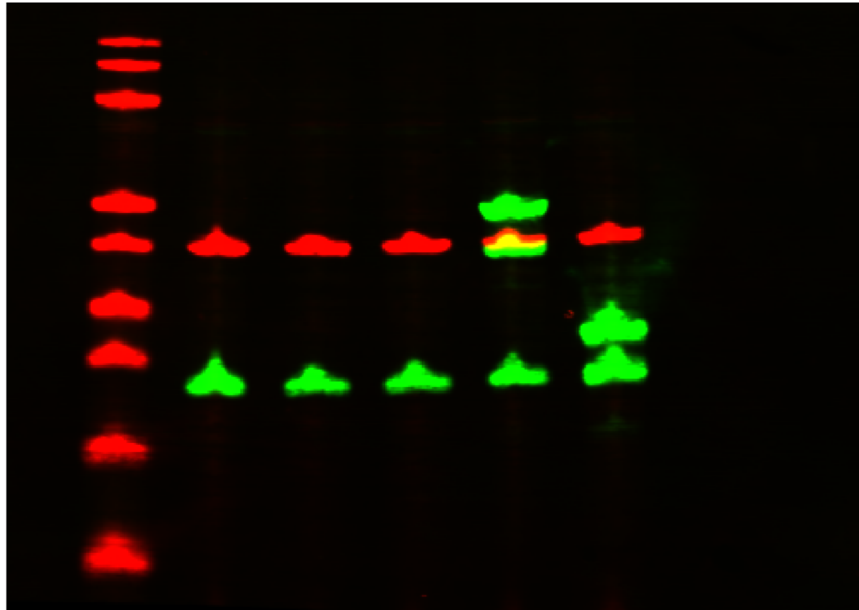

Figure 12. Western blot of SH-SY5Y using anti-β-actin and anti-DJ-1. From the left (1) ladder, (2) uninfected SH-SY5Y, (3) empty mCherry, (4) empty 3x FLAG, (5) mCherry-DJ-1, (6) 3x FLAG-DJ-1.

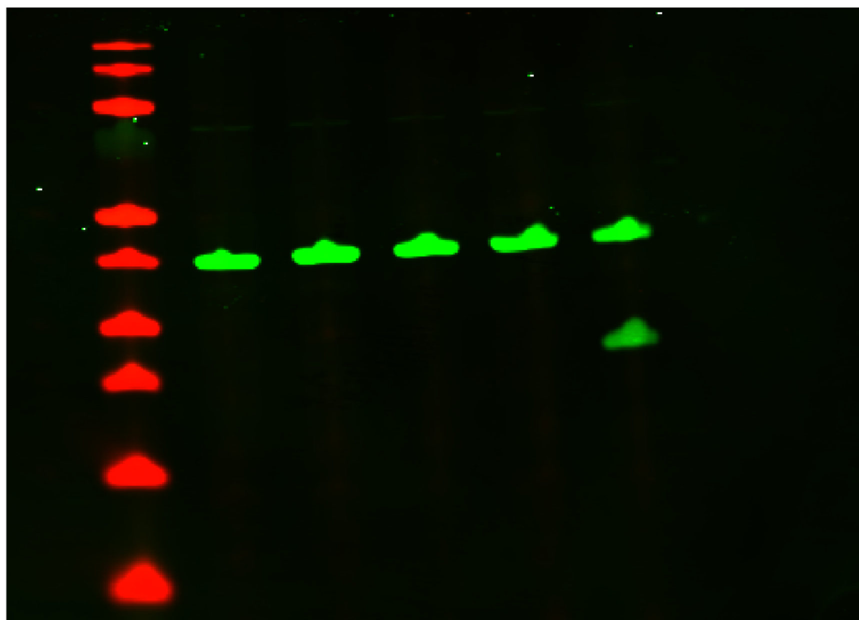

Figure 13. Western blot of SH-SY5Y using anti-β-actin and anti-FLAG. From the left (1) ladder, (2) uninfected SH-SY5Y, (3) empty mCherry, (4) empty 3x FLAG, (5) mCherry-DJ-1, (6) 3x FLAG-DJ-1.

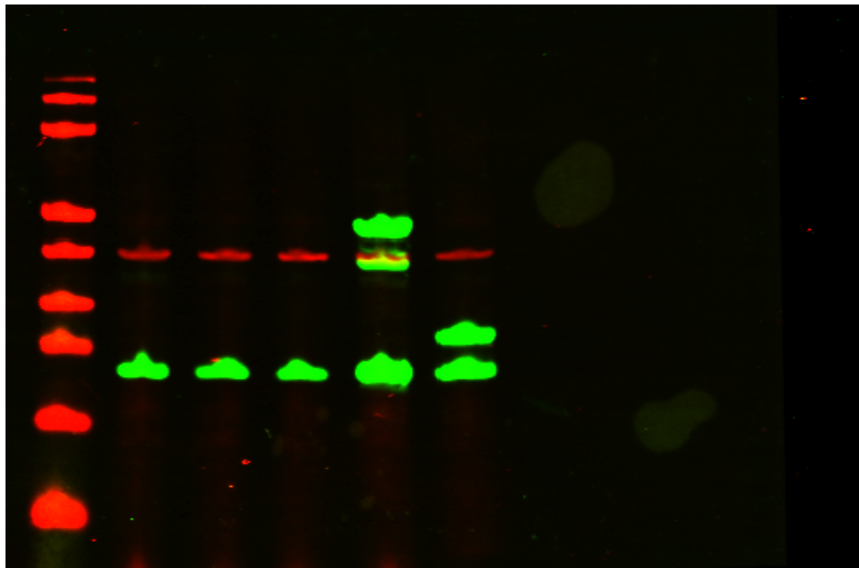

Figure 14. Western blot of M17 using anti-β-actin and anti-DJ-1. From the left (1) ladder, (2) uninfected M17, (3) empty mCherry, (4) empty 3x FLAG, (5) mCherry-DJ-1, (6) 3x FLAG-DJ-1.

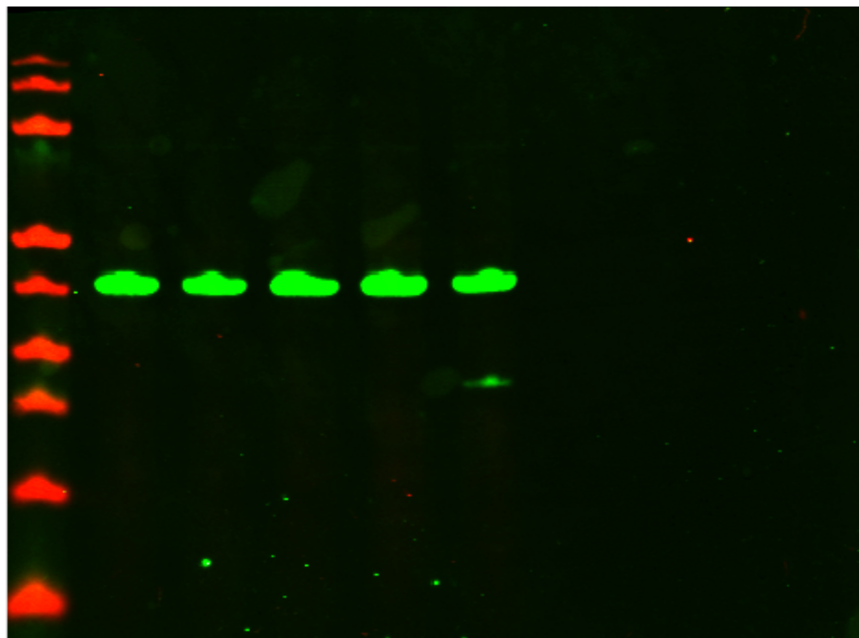

Figure 15. Western blot of M17 using anti-β-actin and anti-FLAG. From the left (1) ladder, (2) uninfected M17, (3) empty mCherry, (4) empty 3x FLAG, (5) mCherry-DJ-1, (6) 3x FLAG-DJ-1.

DISCUSSION

As discussed earlier, neuroblastoma cells are cancerous nerve tissue intended to model dopaminergic neurons. However, DJ-1 is found in highest concentration in astrocytes, so why model them in neurons. The majority of DJ-1s known interactors are most highly expressed in neurons, so it is most appropriate to study the function of DJ-1 in an environment that endogenously expresses interactors. For example, Hsp31 is known to modulate α-synuclein aggregation and is able to inhibit fibrilization *in vitro*.[218] Hsp31 levels also increase when exposed to an oxidative stressor, H_2O_2. These behaviors would be expected to be similar in DJ-1, as Hsp31 is a homolog of DJ-1, however in order to create the right conditions, DJ-1 would need to be overexpressed in neurons rather than astrocytes. Furthermore, selective dopaminergic neuron death in the *substantia nigra* is one of the known hallmarks of PD while the role of astrocytes in PD has been less investigated.

In this study, we were interested in making a DJ-1-associated model of Parkinson's disease. Mutant DJ-1_{C106A} was engineered to examine the importance of this particular residue. It is this residue, and its proximate active site, that arguments surrounding the enzymatic activity of DJ-1 are centered. Considering the ubiquitous nature of DJ-1, it is possible that DJ-1 has both chaperone activity, representative of Hsp31 and other known homolog functions, as well as catalytic activity, suggested by the putative active site. However, further experimentation is needed to either confirm or deny this hypothesis.

The models I have created will be used in the lab for future studies. The first of which being a cell viability, or MTT, assay. This will allow us to determine the appropriate concentration of oxidative stress to expose cells to in order to simulate levels of oxidative stress seen in PD. Next, cells will be exposed to the optimized concentration of oxidative stress and affixed to slips and visualized via confocal microscopy utilizing the mCherry fluorescent tag. This will allow us to examine subcellular localization of DJ-1 under oxidative stress. Next, cells will be exposed again to oxidative stress and subjected to a pull-down assay. DJ-1 will be purified and mass spectrometry will be conducted to identify any potential interactors.

There are a number of reasons for which we believe that exposure to oxidative stress may cause interactions with DJ-1. One thought involves the documented localization of DJ-1 to the mitochondria. As stated earlier, DJ-1 has no mitochondrial targeting sequence so it is unclear why or how DJ-1 localizes to the mitochondria. It is, thus, possible that DJ-1 may be interacting with a protein with a mitochondrial targeting sequence under oxidative conditions leading DJ-1 to localize with interactor. A different line of thinking is focused on the role of the C106 residue specifically. Mutational analysis of cysteine residues in DJ-1 showed that C106 is the most redox-sensitive when mutated to C106A demonstrating diminished oxidation and decreased ability to deter cell death. This mutation has also been shown to bind an outer mitochondrial membrane protein far less than *wt* DJ-1 suggesting that the ability of the C106 residue to be oxidized may be needed for the localization of DJ-1 to the mitochondria. However, this study brings up an interesting question. Is it possible that the C106A mutant binds

less because it lacks the ability to be oxidized or because it has already bound another interactor and the functional ability of the original cysteine residue to bind and release is now extinguished by mutating the 106 residue.

It is clear that elucidating the function of DJ-1 will not be a simple, linear progression. It is my hope that the constructs I made in order to model DJ-1 associated Parkinson's disease will help to further progress in order to better understand the function of DJ-1 in PD.

REFERENCES

1. Lang AE, Lozano AM. Parkinson's disease. Second of two parts. N Engl J Med 1998;339:1130-43.
2. Lang AE, Lozano AM. Parkinson's disease. First of two parts. N Engl J Med 1998;339:1044-53.
3. de Rijk MC, Tzourio C, Breteler MM, et al. Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. J Neurol Neurosurg Psychiatry 1997;62:10-5.
4. Van Den Eeden SK, Tanner CM, Bernstein AL, et al. Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. Am J Epidemiol 2003;157:1015-22.
5. Parkinson J. An essay on the shaking palsy. 1817. J Neuropsychiatry Clin Neurosci 2002;14:223-36; discussion 2.
6. Gowers WR. A Manual of the Diseases of the Nervous System. Vol. I. Diseases of the Nerves and Spinal Cord. Philadelphia1900.
7. Bell J, Clark AJ. A pedigree of paralysis agitans. Ann Eugen 1926:455-62.
8. Ward CD, Duvoisin RC, Ince SE, Nutt JD, Eldridge R, Calne DB. Parkinson's disease in 65 pairs of twins and in a set of quadruplets. Neurology 1983;33:815-24.
9. Marttila RJ, Kaprio J, Koskenvuo M, Rinne UK. Parkinson's disease in a nationwide twin cohort. Neurology 1988;38:1217-9.
10. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983;219:979-80.
11. Marder K, Tang MX, Mejia H, et al. Risk of Parkinson's disease among first-degree relatives: A community-based study. Neurology 1996;47:155-60.
12. Polymeropoulos MH, Higgins JJ, Golbe LI, et al. Mapping of a gene for Parkinson's disease to chromosome 4q21-q23. Science 1996;274:1197-9.
13. Muthane UB, Swamy HS, Satishchandra P, Subhash MN, Rao S, Subbakrishna D. Early onset Parkinson's disease: are juvenile- and young-onset different? Mov Disord 1994;9:539-44.
14. Ayano G. Parkinson's Disease: A Concise Overview of Etiology, Epidemiology, Diagnosis, Comorbidity and Management. J Neurol Disord 2016;4.
15. Ylikotila P, Tiirikka T, Moilanen JS, Kaariainen H, Marttila R, Majamaa K. Epidemiology of early-onset Parkinson's disease in Finland. Parkinsonism Relat Disord 2015;21:938-42.
16. Savica R, Grossardt BR, Bower JH, Ahlskog JE, Rocca WA. Time Trends in the Incidence of Parkinson Disease. JAMA Neurol 2016;73:981-9.
17. Lucking CB, Durr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. N Engl J Med 2000;342:1560-7.
18. Pankratz N, Foroud T. Genetics of Parkinson disease. Genet Med 2007;9:801-11.
19. Clark LN, Wang Y, Karlins E, et al. Frequency of LRRK2 mutations in early- and late-onset Parkinson disease. Neurology 2006;67:1786-91.
20. Symptoms. 2017. at http://www.pdf.org/symptoms.)

21. Parkinson’s Disease: Symptoms. 2015. at http://www.mayoclinic.org/diseases-conditions/parkinsons-disease/basics/symptoms/con-20028488.)
22. Sethi K. Levodopa unresponsive symptoms in Parkinson disease. Mov Disord 2008;23 Suppl 3:S521-33.
23. Parkinson’s Disease Foundation. Statistics on Parkinson’s. 2017.
24. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988;51:745-52.
25. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197-211.
26. Braak H, Del Tredici K. Neuropathological Staging of Brain Pathology in Sporadic Parkinson's disease: Separating the Wheat from the Chaff. J Parkinsons Dis 2017;7:S73-S87.
27. Beyer K, Domingo-Sabat M, Ariza A. Molecular pathology of Lewy body diseases. Int J Mol Sci 2009;10:724-45.
28. Tai HC, Schuman EM. Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction. Nat Rev Neurosci 2008;9:826-38.
29. McNaught KS, Belizaire R, Isacson O, Jenner P, Olanow CW. Altered proteasomal function in sporadic Parkinson's disease. Exp Neurol 2003;179:38-46.
30. McNaught KS, Jenner P. Proteasomal function is impaired in substantia nigra in Parkinson's disease. Neurosci Lett 2001;297:191-4.
31. Tofaris GK, Razzaq A, Ghetti B, Lilley KS, Spillantini MG. Ubiquitination of alpha-synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. J Biol Chem 2003;278:44405-11.
32. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 1991;114 (Pt 5):2283-301.
33. Zecca L, Tampellini D, Gerlach M, Riederer P, Fariello RG, Sulzer D. Substantia nigra neuromelanin: structure, synthesis, and molecular behaviour. Mol Pathol 2001;54:414-8.
34. Klein C, Westenberger A. Genetics of Parkinson's disease. Cold Spring Harb Perspect Med 2012;2:a008888.
35. Eichler EE, Flint J, Gibson G, et al. Missing heritability and strategies for finding the underlying causes of complex disease. Nat Rev Genet 2010;11:446-50.
36. Gasser T, Muller-Myhsok B, Wszolek ZK, et al. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nat Genet 1998;18:262-5.
37. Jankovic J, McDermott M, Carter J, et al. Variable expression of Parkinson's disease: a base-line analysis of the DATATOP cohort. The Parkinson Study Group. Neurology 1990;40:1529-34.
38. Zhu M, Zhao S. Candidate gene identification approach: progress and challenges. Int J Biol Sci 2007;3:420-7.
39. Wilkening S, Chen B, Bermejo JL, Canzian F. Is there still a need for candidate gene approaches in the era of genome-wide association studies? Genomics 2009;93:415-9.

40. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045-7.
41. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605-8.
42. Singleton AB, Farrer M, Johnson J, et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science 2003;302:841.
43. Leroy E, Boyer R, Auburger G, et al. The ubiquitin pathway in Parkinson's disease. Nature 1998;395:451-2.
44. Valente EM, Abou-Sleiman PM, Caputo V, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004;304:1158-60.
45. Bonifati V, Rizzu P, van Baren MJ, et al. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 2003;299:256-9.
46. Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F. A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. Ann Neurol 2002;51:296-301.
47. Ramirez A, Heimbach A, Grundemann J, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet 2006;38:1184-91.
48. Hicks AA, Petursson H, Jonsson T, et al. A susceptibility gene for late-onset idiopathic Parkinson's disease. Ann Neurol 2002;52:549-55.
49. Pankratz N, Nichols WC, Uniacke SK, et al. Significant linkage of Parkinson disease to chromosome 2q36-37. Am J Hum Genet 2003;72:1053-7.
50. Pankratz N, Nichols WC, Uniacke SK, et al. Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. Am J Hum Genet 2002;71:124-35.
51. Pankratz N, Nichols WC, Uniacke SK, et al. Genome-wide linkage analysis and evidence of gene-by-gene interactions in a sample of 362 multiplex Parkinson disease families. Hum Mol Genet 2003;12:2599-608.
52. Scott WK, Nance MA, Watts RL, et al. Complete genomic screen in Parkinson disease: evidence for multiple genes. JAMA 2001;286:2239-44.
53. Strauss KM, Martins LM, Plun-Favreau H, et al. Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. Hum Mol Genet 2005;14:2099-111.
54. Shi CH, Tang BS, Wang L, et al. PLA2G6 gene mutation in autosomal recessive early-onset parkinsonism in a Chinese cohort. Neurology 2011;77:75-81.
55. Di Fonzo A, Dekker MC, Montagna P, et al. FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. Neurology 2009;72:240-5.
56. Satake W, Nakabayashi Y, Mizuta I, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 2009;41:1303-7.
57. Wider C, Skipper L, Solida A, et al. Autosomal dominant dopa-responsive parkinsonism in a multigenerational Swiss family. Parkinsonism Relat Disord 2008;14:465-70.
58. Chartier-Harlin MC, Dachsel JC, Vilarino-Guell C, et al. Translation initiator EIF4G1 mutations in familial Parkinson disease. Am J Hum Genet 2011;89:398-406.

59. Edvardson S, Cinnamon Y, Ta-Shma A, et al. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. PLoS One 2012;7:e36458.
60. Krebs CE, Karkheiran S, Powell JC, et al. The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive Parkinsonism with generalized seizures. Hum Mutat 2013;34:1200-7.
61. Vilarino-Guell C, Rajput A, Milnerwood AJ, et al. DNAJC13 mutations in Parkinson disease. Hum Mol Genet 2014;23:1794-801.
62. Lesage S, Drouet V, Majounie E, et al. Loss of VPS13C Function in Autosomal-Recessive Parkinsonism Causes Mitochondrial Dysfunction and Increases PINK1/Parkin-Dependent Mitophagy. Am J Hum Genet 2016;98:500-13.
63. Marx FP, Holzmann C, Strauss KM, et al. Identification and functional characterization of a novel R621C mutation in the synphilin-1 gene in Parkinson's disease. Hum Mol Genet 2003;12:1223-31.
64. Le WD, Xu P, Jankovic J, et al. Mutations in NR4A2 associated with familial Parkinson disease. Nat Genet 2003;33:85-9.
65. Pankratz N, Byder L, Halter C, et al. Presence of an APOE4 allele results in significantly earlier onset of Parkinson's disease and a higher risk with dementia. Mov Disord 2006;21:45-9.
66. Huang X, Chen PC, Poole C. APOE-[epsilon]2 allele associated with higher prevalence of sporadic Parkinson disease. Neurology 2004;62:2198-202.
67. Harhangi BS, de Rijk MC, van Duijn CM, Van Broeckhoven C, Hofman A, Breteler MM. APOE and the risk of PD with or without dementia in a population-based study. Neurology 2000;54:1272-6.
68. Aharon-Peretz J, Rosenbaum H, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson's disease in Ashkenazi Jews. N Engl J Med 2004;351:1972-7.
69. Zabetian CP, Hutter CM, Factor SA, et al. Association analysis of MAPT H1 haplotype and subhaplotypes in Parkinson's disease. Ann Neurol 2007;62:137-44.
70. Rademakers R, Cruts M, van Broeckhoven C. The role of tau (MAPT) in frontotemporal dementia and related tauopathies. Hum Mutat 2004;24:277-95.
71. Deng HX, Shi Y, Yang Y, et al. Identification of TMEM230 mutations in familial Parkinson's disease. Nat Genet 2016;48:733-9.
72. Mancuso M, Filosto M, Oh SJ, DiMauro S. A novel polymerase gamma mutation in a family with ophthalmoplegia, neuropathy, and Parkinsonism. Arch Neurol 2004;61:1777-9.
73. Shi M, Jin J, Wang Y, et al. Mortalin: a protein associated with progression of Parkinson disease? J Neuropathol Exp Neurol 2008;67:117-24.
74. Shi G, Lee JR, Grimes DA, et al. Functional alteration of PARL contributes to mitochondrial dysregulation in Parkinson's disease. Hum Mol Genet 2011;20:1966-74.
75. Chan DK, Lam MK, Wong R, Hung WT, Wilcken DE. Strong association between N-acetyltransferase 2 genotype and PD in Hong Kong Chinese. Neurology 2003;60:1002-5.

76. Pankratz N, Wilk JB, Latourelle JC, et al. Genomewide association study for susceptibility genes contributing to familial Parkinson disease. Hum Genet 2009;124:593-605.
77. Hamza TH, Zabetian CP, Tenesa A, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. Nat Genet 2010;42:781-5.
78. Kruger R, Kuhn W, Muller T, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 1998;18:106-8.
79. Zarranz JJ, Alegre J, Gomez-Esteban JC, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 2004;55:164-73.
80. Giasson BI, Duda JE, Murray IV, et al. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science 2000;290:985-9.
81. Irizarry MC, Kim TW, McNamara M, et al. Characterization of the precursor protein of the non-A beta component of senile plaques (NACP) in the human central nervous system. J Neuropathol Exp Neurol 1996;55:889-95.
82. Kahle PJ, Neumann M, Ozmen L, et al. Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha -synuclein in human and transgenic mouse brain. J Neurosci 2000;20:6365-73.
83. Clayton DF, George JM. The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. Trends Neurosci 1998;21:249-54.
84. Devi L, Raghavendran V, Prabhu BM, Avadhani NG, Anandatheerthavarada HK. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. J Biol Chem 2008;283:9089-100.
85. Uversky VN. A protein-chameleon: conformational plasticity of alpha-synuclein, a disordered protein involved in neurodegenerative disorders. J Biomol Struct Dyn 2003;21:211-34.
86. Hashimoto M, Masliah E. Alpha-synuclein in Lewy body disease and Alzheimer's disease. Brain Pathol 1999;9:707-20.
87. Lucking CB, Brice A. Alpha-synuclein and Parkinson's disease. Cell Mol Life Sci 2000;57:1894-908.
88. da Costa CA, Ancolio K, Checler F. Wild-type but not Parkinson's disease-related ala-53 --> Thr mutant alpha -synuclein protects neuronal cells from apoptotic stimuli. J Biol Chem 2000;275:24065-9.
89. Alves Da Costa C, Paitel E, Vincent B, Checler F. Alpha-synuclein lowers p53-dependent apoptotic response of neuronal cells. Abolishment by 6-hydroxydopamine and implication for Parkinson's disease. J Biol Chem 2002;277:50980-4.
90. Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, Goedert M. Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. Neurosci Lett 1998;251:205-8.
91. Conway KA, Harper JD, Lansbury PT. Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. Nat Med 1998;4:1318-20.

92. Snyder H, Mensah K, Theisler C, Lee J, Matouschek A, Wolozin B. Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. J Biol Chem 2003;278:11753-9.
93. Lindersson E, Beedholm R, Hojrup P, et al. Proteasomal inhibition by alpha-synuclein filaments and oligomers. J Biol Chem 2004;279:12924-34.
94. Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM. Degradation of alpha-synuclein by proteasome. J Biol Chem 1999;274:33855-8.
95. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. Alpha-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem 2003;278:25009-13.
96. Conway KA, Lee SJ, Rochet JC, Ding TT, Williamson RE, Lansbury PT, Jr. Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci U S A 2000;97:571-6.
97. Sharon R, Bar-Joseph I, Frosch MP, Walsh DM, Hamilton JA, Selkoe DJ. The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. Neuron 2003;37:583-95.
98. Masliah E, Rockenstein E, Veinbergs I, et al. beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci U S A 2001;98:12245-50.
99. Rideout HJ, Dietrich P, Wang Q, Dauer WT, Stefanis L. alpha-synuclein is required for the fibrillar nature of ubiquitinated inclusions induced by proteasomal inhibition in primary neurons. J Biol Chem 2004;279:46915-20.
100. McNaught KS, Perl DP, Brownell AL, Olanow CW. Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. Ann Neurol 2004;56:149-62.
101. Matsumine H, Saito M, Shimoda-Matsubayashi S, et al. Localization of a gene for an autosomal recessive form of juvenile Parkinsonism to chromosome 6q25.2-27. Am J Hum Genet 1997;60:588-96.
102. West AB, Maidment NT. Genetics of parkin-linked disease. Hum Genet 2004;114:327-36.
103. Mata IF, Lockhart PJ, Farrer MJ. Parkin genetics: one model for Parkinson's disease. Hum Mol Genet 2004;13 Spec No 1:R127-33.
104. Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM. Parkin functions as an E2-dependent ubiquitin- protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. Proc Natl Acad Sci U S A 2000;97:13354-9.
105. Shimura H, Hattori N, Kubo S, et al. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 2000;25:302-5.
106. Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y, Takahashi R. An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. Cell 2001;105:891-902.
107. Palacino JJ, Sagi D, Goldberg MS, et al. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J Biol Chem 2004;279:18614-22.

108. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. Proc Natl Acad Sci U S A 2003;100:4078-83.
109. Darios F, Corti O, Lucking CB, et al. Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. Hum Mol Genet 2003;12:517-26.
110. Petrucelli L, O'Farrell C, Lockhart PJ, et al. Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. Neuron 2002;36:1007-19.
111. Yang Y, Nishimura I, Imai Y, Takahashi R, Lu B. Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in Drosophila. Neuron 2003;37:911-24.
112. Valente EM, Bentivoglio AR, Dixon PH, et al. Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am J Hum Genet 2001;68:895-900.
113. Valente EM, Brancati F, Ferraris A, et al. PARK6-linked parkinsonism occurs in several European families. Ann Neurol 2002;51:14-8.
114. Valente EM, Salvi S, Ialongo T, et al. PINK1 mutations are associated with sporadic early-onset parkinsonism. Ann Neurol 2004;56:336-41.
115. Bonifati V, Rohe CF, Breedveld GJ, et al. Early-onset parkinsonism associated with PINK1 mutations: frequency, genotypes, and phenotypes. Neurology 2005;65:87-95.
116. Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ. The PINK1/Parkin pathway regulates mitochondrial morphology. Proc Natl Acad Sci U S A 2008;105:1638-43.
117. Narendra DP, Youle RJ. Targeting mitochondrial dysfunction: role for PINK1 and Parkin in mitochondrial quality control. Antioxid Redox Signal 2011;14:1929-38.
118. Brice A. Genetics of Parkinson's disease: LRRK2 on the rise. Brain 2005;128:2760-2.
119. Giasson BI, Covy JP, Bonini NM, et al. Biochemical and pathological characterization of Lrrk2. Ann Neurol 2006;59:315-22.
120. Gandhi PN, Wang X, Zhu X, Chen SG, Wilson-Delfosse AL. The Roc domain of leucine-rich repeat kinase 2 is sufficient for interaction with microtubules. J Neurosci Res 2008;86:1711-20.
121. Paisan-Ruiz C, Jain S, Evans EW, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 2004;44:595-600.
122. Nuytemans K, Theuns J, Cruts M, Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. Hum Mutat 2010;31:763-80.
123. MacLeod D, Dowman J, Hammond R, Leete T, Inoue K, Abeliovich A. The familial Parkinsonism gene LRRK2 regulates neurite process morphology. Neuron 2006;52:587-93.
124. Maraganore DM, Farrer MJ, Hardy JA, Lincoln SJ, McDonnell SK, Rocca WA. Case-control study of the ubiquitin carboxy-terminal hydrolase L1 gene in Parkinson's disease. Neurology 1999;53:1858-60.

125. Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, Pohl J. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science 1989;246:670-3.
126. Osaka H, Wang YL, Takada K, et al. Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. Hum Mol Genet 2003;12:1945-58.
127. Liu Y, Fallon L, Lashuel HA, Liu Z, Lansbury PT, Jr. The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. Cell 2002;111:209-18.
128. Lowe J, McDermott H, Landon M, Mayer RJ, Wilkinson KD. Ubiquitin carboxyl-terminal hydrolase (PGP 9.5) is selectively present in ubiquitinated inclusion bodies characteristic of human neurodegenerative diseases. J Pathol 1990;161:153-60.
129. Healy DG, Abou-Sleiman PM, Casas JP, et al. UCHL-1 is not a Parkinson's disease susceptibility gene. Ann Neurol 2006;59:627-33.
130. Healy DG, Abou-Sleiman PM, Wood NW. Genetic causes of Parkinson's disease: UCHL-1. Cell Tissue Res 2004;318:189-94.
131. van Duijn CM, Dekker MCJ, Bonifati V, et al. PARK7, a Novel Locus for Autosomal Recessive Early-Onset Parkinsonism, on Chromosome 1p36. American Journal of Human Genetics 2001:629-34.
132. Bonifati V, Oostra BA, Heutink P. Linking DJ-1 to neurodegeneration offers novel insights for understanding the pathogenesis of Parkinson's disease. J Mol Med (Berl) 2004;82:163-74.
133. Lockhart PJ, Lincoln S, Hulihan M, et al. DJ-1 mutations are a rare cause of recessively inherited early onset parkinsonism mediated by loss of protein function. J Med Genet 2004;41:e22.
134. Pankratz N, Pauciulo MW, Elsaesser VE, et al. Mutations in DJ-1 are rare in familial Parkinson disease. Neurosci Lett 2006;408:209-13.
135. Bandyopadhyay S, Cookson MR. Evolutionary and functional relationships within the DJ1 superfamily. BMC Evol Biol 2004;4:6.
136. Kotaria N, Hinz U, Zechel S, von Bohlen Und Halbach O. Localization of DJ-1 protein in the murine brain. Cell Tissue Res 2005;322:503-7.
137. Kumaran R, Vandrovcova J, Luk C, et al. Differential DJ-1 gene expression in Parkinson's disease. Neurobiol Dis 2009;36:393-400.
138. Canet-Aviles RM, Wilson MA, Miller DW, et al. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. Proc Natl Acad Sci U S A 2004;101:9103-8.
139. Trudler D, Weinreb O, Mandel SA, Youdim MB, Frenkel D. DJ-1 deficiency triggers microglia sensitivity to dopamine toward a pro-inflammatory phenotype that is attenuated by rasagiline. J Neurochem 2014;129:434-47.
140. Bandopadhyay R, Kingsbury AE, Cookson MR, et al. The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. Brain 2004;127:420-30.
141. Booth HDE, Hirst WD, Wade-Martins R. The Role of Astrocyte Dysfunction in Parkinson's Disease Pathogenesis. Trends Neurosci 2017;40:358-70.

142. Neumann M, Muller V, Gorner K, Kretzschmar HA, Haass C, Kahle PJ. Pathological properties of the Parkinson's disease-associated protein DJ-1 in alpha-synucleinopathies and tauopathies: relevance for multiple system atrophy and Pick's disease. Acta Neuropathol 2004;107:489-96.
143. Larsen NJ, Ambrosi G, Mullett SJ, Berman SB, Hinkle DA. DJ-1 knock-down impairs astrocyte mitochondrial function. Neuroscience 2011;196:251-64.
144. Mullett SJ, Di Maio R, Greenamyre JT, Hinkle DA. DJ-1 expression modulates astrocyte-mediated protection against neuronal oxidative stress. J Mol Neurosci 2013;49:507-11.
145. Ashley AK, Hinds AI, Hanneman WH, Tjalkens RB, Legare ME. DJ-1 mutation decreases astroglial release of inflammatory mediators. Neurotoxicology 2016;52:198-203.
146. Nagakubo D, Taira T, Kitaura H, et al. DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras. Biochem Biophys Res Commun 1997;231:509-13.
147. Le Naour F, Misek DE, Krause MC, et al. Proteomics-based identification of RS/DJ-1 as a novel circulating tumor antigen in breast cancer. Clin Cancer Res 2001;7:3328-35.
148. Pardo M, Garcia A, Thomas B, et al. The characterization of the invasion phenotype of uveal melanoma tumour cells shows the presence of MUC18 and HMG-1 metastasis markers and leads to the identification of DJ-1 as a potential serum biomarker. Int J Cancer 2006;119:1014-22.
149. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol 2012;13:283-96.
150. Inestrosa NC, Arenas E. Emerging roles of Wnts in the adult nervous system. Nat Rev Neurosci 2010;11:77-86.
151. Wagenfeld A, Gromoll J, Cooper TG. Molecular cloning and expression of rat contraception associated protein 1 (CAP1), a protein putatively involved in fertilization. Biochem Biophys Res Commun 1998;251:545-9.
152. Takahashi K, Taira T, Niki T, Seino C, Iguchi-Ariga SM, Ariga H. DJ-1 positively regulates the androgen receptor by impairing the binding of PIASx alpha to the receptor. J Biol Chem 2001;276:37556-63.
153. Hod Y, Pentyala SN, Whyard TC, El-Maghrabi MR. Identification and characterization of a novel protein that regulates RNA-protein interaction. J Cell Biochem 1999;72:435-44.
154. Shendelman S, Jonason A, Martinat C, Leete T, Abeliovich A. DJ-1 is a redox-dependent molecular chaperone that inhibits alpha-synuclein aggregate formation. PLoS Biol 2004;2:e362.
155. Andres-Mateos E, Perier C, Zhang L, et al. DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. Proc Natl Acad Sci U S A 2007;104:14807-12.
156. Zondler L, Miller-Fleming L, Repici M, et al. DJ-1 interactions with alpha-synuclein attenuate aggregation and cellular toxicity in models of Parkinson's disease. Cell Death Dis 2014;5:e1350.

157. Mitsumoto A, Nakagawa Y. DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin. Free Radic Res 2001;35:885-93.
158. Mitsumoto A, Nakagawa Y, Takeuchi A, Okawa K, Iwamatsu A, Takanezawa Y. Oxidized forms of peroxiredoxins and DJ-1 on two-dimensional gels increased in response to sublethal levels of paraquat. Free Radic Res 2001;35:301-10.
159. Taira T, Saito Y, Niki T, Iguchi-Ariga SM, Takahashi K, Ariga H. DJ-1 has a role in antioxidative stress to prevent cell death. EMBO Rep 2004;5:213-8.
160. Maita C, Maita H, Iguchi-Ariga SM, Ariga H. Monomer DJ-1 and its N-terminal sequence are necessary for mitochondrial localization of DJ-1 mutants. PLoS One 2013;8:e54087.
161. Zhang L, Shimoji M, Thomas B, et al. Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. Hum Mol Genet 2005;14:2063-73.
162. Junn E, Jang WH, Zhao X, Jeong BS, Mouradian MM. Mitochondrial localization of DJ-1 leads to enhanced neuroprotection. J Neurosci Res 2009;87:123-9.
163. Moore DJ, Zhang L, Troncoso J, et al. Association of DJ-1 and parkin mediated by pathogenic DJ-1 mutations and oxidative stress. Hum Mol Genet 2005;14:71-84.
164. Joselin AP, Hewitt SJ, Callaghan SM, et al. ROS-dependent regulation of Parkin and DJ-1 localization during oxidative stress in neurons. Hum Mol Genet 2012;21:4888-903.
165. Tang B, Xiong H, Sun P, et al. Association of PINK1 and DJ-1 confers digenic inheritance of early-onset Parkinson's disease. Hum Mol Genet 2006;15:1816-25.
166. Hao LY, Giasson BI, Bonini NM. DJ-1 is critical for mitochondrial function and rescues PINK1 loss of function. Proc Natl Acad Sci U S A 2010;107:9747-52.
167. Thomas KJ, McCoy MK, Blackinton J, et al. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. Hum Mol Genet 2011;20:40-50.
168. Batelli S, Albani D, Rametta R, et al. DJ-1 modulates alpha-synuclein aggregation state in a cellular model of oxidative stress: relevance for Parkinson's disease and involvement of HSP70. PLoS One 2008;3:e1884.
169. Yokota T, Sugawara K, Ito K, Takahashi R, Ariga H, Mizusawa H. Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. Biochem Biophys Res Commun 2003;312:1342-8.
170. Wickremaratchi MM, Ben-Shlomo Y, Morris HR. The effect of onset age on the clinical features of Parkinson's disease. Eur J Neurol 2009;16:450-6.
171. Schrag A, Schott JM. Epidemiological, clinical, and genetic characteristics of early-onset parkinsonism. Lancet Neurol 2006;5:355-63.
172. Wickremaratchi MM, Knipe MD, Sastry BS, et al. The motor phenotype of Parkinson's disease in relation to age at onset. Mov Disord 2011;26:457-63.
173. Peran P, Cherubini A, Assogna F, et al. Magnetic resonance imaging markers of Parkinson's disease nigrostriatal signature. Brain 2010;133:3423-33.
174. Cha SS, Jung HI, Jeon H, et al. Crystal structure of filamentous aggregates of human DJ-1 formed in an inorganic phosphate-dependent manner. J Biol Chem 2008;283:34069-75.

175. Rizzu P, Hinkle DA, Zhukareva V, et al. DJ-1 colocalizes with tau inclusions: a link between parkinsonism and dementia. Ann Neurol 2004;55:113-8.
176. Baulac S, LaVoie MJ, Strahle J, Schlossmacher MG, Xia W. Dimerization of Parkinson's disease-causing DJ-1 and formation of high molecular weight complexes in human brain. Mol Cell Neurosci 2004;27:236-46.
177. Moore DJ, Zhang L, Dawson TM, Dawson VL. A missense mutation (L166P) in DJ-1, linked to familial Parkinson's disease, confers reduced protein stability and impairs homo-oligomerization. J Neurochem 2003;87:1558-67.
178. Miller DW, Ahmad R, Hague S, et al. L166P mutant DJ-1, causative for recessive Parkinson's disease, is degraded through the ubiquitin-proteasome system. J Biol Chem 2003;278:36588-95.
179. Olzmann JA, Brown K, Wilkinson KD, et al. Familial Parkinson's disease-associated L166P mutation disrupts DJ-1 protein folding and function. J Biol Chem 2004;279:8506-15.
180. Guo JF, Xiao B, Liao B, et al. Mutation analysis of Parkin, PINK1, DJ-1 and ATP13A2 genes in Chinese patients with autosomal recessive early-onset Parkinsonism. Mov Disord 2008;23:2074-9.
181. Ramsey CP, Giasson BI. L10p and P158DEL DJ-1 mutations cause protein instability, aggregation, and dimerization impairments. J Neurosci Res 2010;88:3111-24.
182. Abou-Sleiman PM, Healy DG, Quinn N, Lees AJ, Wood NW. The role of pathogenic DJ-1 mutations in Parkinson's disease. Ann Neurol 2003;54:283-6.
183. Hering R, Strauss KM, Tao X, et al. Novel homozygous p.E64D mutation in DJ1 in early onset Parkinson disease (PARK7). Hum Mutat 2004;24:321-9.
184. Hedrich K, Djarmati A, Schafer N, et al. DJ-1 (PARK7) mutations are less frequent than Parkin (PARK2) mutations in early-onset Parkinson disease. Neurology 2004;62:389-94.
185. Hedrich K, Schafer N, Hering R, et al. The R98Q variation in DJ-1 represents a rare polymorphism. Ann Neurol 2004;55:145; author reply -6.
186. Hague S, Rogaeva E, Hernandez D, et al. Early-onset Parkinson's disease caused by a compound heterozygous DJ-1 mutation. Ann Neurol 2003;54:271-4.
187. Ben-Nissan G, Chotiner A, Tarnavsky M, Sharon M. Structural Characterization of Missense Mutations Using High Resolution Mass Spectrometry: A Case Study of the Parkinson's-Related Protein, DJ-1. J Am Soc Mass Spectrom 2016;27:1062-70.
188. Annesi G, Savettieri G, Pugliese P, et al. DJ-1 mutations and parkinsonism-dementia-amyotrophic lateral sclerosis complex. Ann Neurol 2005;58:803-7.
189. Ramsey CP, Giasson BI. The E163K DJ-1 mutant shows specific antioxidant deficiency. Brain Res 2008;1239:1-11.
190. Macedo MG, Verbaan D, Fang Y, et al. Genotypic and phenotypic characteristics of Dutch patients with early onset Parkinson's disease. Mov Disord 2009;24:196-203.
191. Wilson MA, Collins JL, Hod Y, Ringe D, Petsko GA. The 1.1-A resolution crystal structure of DJ-1, the protein mutated in autosomal recessive early onset Parkinson's disease. Proc Natl Acad Sci U S A 2003;100:9256-61.
192. Lee SJ, Kim SJ, Kim IK, et al. Crystal structures of human DJ-1 and Escherichia coli Hsp31, which share an evolutionarily conserved domain. J Biol Chem 2003;278:44552-9.

193. Tao X, Tong L. Crystal structure of human DJ-1, a protein associated with early onset Parkinson's disease. J Biol Chem 2003;278:31372-9.
194. Huai Q, Sun Y, Wang H, et al. Crystal structure of DJ-1/RS and implication on familial Parkinson's disease. FEBS Lett 2003;549:171-5.
195. Chen J, Li L, Chin LS. Parkinson disease protein DJ-1 converts from a zymogen to a protease by carboxyl-terminal cleavage. Hum Mol Genet 2010;19:2395-408.
196. Barford D. The role of cysteine residues as redox-sensitive regulatory switches. Curr Opin Struct Biol 2004;14:679-86.
197. Kinumi T, Kimata J, Taira T, Ariga H, Niki E. Cysteine-106 of DJ-1 is the most sensitive cysteine residue to hydrogen peroxide-mediated oxidation in vivo in human umbilical vein endothelial cells. Biochem Biophys Res Commun 2004;317:722-8.
198. Ren H, Fu K, Wang D, Mu C, Wang G. Oxidized DJ-1 interacts with the mitochondrial protein BCL-XL. J Biol Chem 2011;286:35308-17.
199. Wilson MA, St Amour CV, Collins JL, Ringe D, Petsko GA. The 1.8-A resolution crystal structure of YDR533Cp from Saccharomyces cerevisiae: a member of the DJ-1/ThiJ/PfpI superfamily. Proc Natl Acad Sci U S A 2004;101:1531-6.
200. Girotto S, Cendron L, Bisaglia M, et al. DJ-1 is a copper chaperone acting on SOD1 activation. J Biol Chem 2014;289:10887-99.
201. Lakshminarasimhan M, Maldonado MT, Zhou W, Fink AL, Wilson MA. Structural impact of three Parkinsonism-associated missense mutations on human DJ-1. Biochemistry 2008;47:1381-92.
202. Orth M, Tabrizi SJ. Models of Parkinson's disease. Mov Disord 2003;18:729-37.
203. Lopes FM, Schroder R, da Frota ML, Jr., et al. Comparison between proliferative and neuron-like SH-SY5Y cells as an in vitro model for Parkinson disease studies. Brain Res 2010;1337:85-94.
204. Xie HR, Hu LS, Li GY. SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. Chin Med J (Engl) 2010;123:1086-92.
205. Filograna R, Civiero L, Ferrari V, et al. Analysis of the Catecholaminergic Phenotype in Human SH-SY5Y and BE(2)-M17 Neuroblastoma Cell Lines upon Differentiation. PLoS One 2015;10:e0136769.
206. Kovalevich J, Langford D. Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. Methods Mol Biol 2013;1078:9-21.
207. Xicoy H, Wieringa B, Martens GJ. The SH-SY5Y cell line in Parkinson's disease research: a systematic review. Mol Neurodegener 2017;12:10.
208. Andres D, Keyser BM, Petrali J, et al. Morphological and functional differentiation in BE(2)-M17 human neuroblastoma cells by treatment with Trans-retinoic acid. BMC Neurosci 2013;14:49.
209. Ciccarone V, Spengler BA, Meyers MB, Biedler JL, Ross RA. Phenotypic diversification in human neuroblastoma cells: expression of distinct neural crest lineages. Cancer Res 1989;49:219-25.
210. Somayajulu-Nitu M, Sandhu JK, Cohen J, et al. Paraquat induces oxidative stress, neuronal loss in substantia nigra region and parkinsonism in adult rats: neuroprotection and amelioration of symptoms by water-soluble formulation of coenzyme Q10. BMC Neurosci 2009;10:88.

211. Wang W, Nguyen LT, Burlak C, et al. Caspase-1 causes truncation and aggregation of the Parkinson's disease-associated protein alpha-synuclein. Proc Natl Acad Sci U S A 2016;113:9587-92.
212. Criddle DN, Gillies S, Baumgartner-Wilson HK, et al. Menadione-induced reactive oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells. J Biol Chem 2006;281:40485-92.
213. Cockrell AS, Kafri T. Gene delivery by lentivirus vectors. Mol Biotechnol 2007;36:184-204.
214. Gropp M, Itsykson P, Singer O, et al. Stable genetic modification of human embryonic stem cells by lentiviral vectors. Mol Ther 2003;7:281-7.
215. Dull T, Zufferey R, Kelly M, et al. A third-generation lentivirus vector with a conditional packaging system. J Virol 1998;72:8463-71.
216. DuBridge RB, Tang P, Hsia HC, Leong PM, Miller JH, Calos MP. Analysis of mutation in human cells by using an Epstein-Barr virus shuttle system. Mol Cell Biol 1987;7:379-87.
217. Pear WS, Nolan GP, Scott ML, Baltimore D. Production of high-titer helper-free retroviruses by transient transfection. Proc Natl Acad Sci U S A 1993;90:8392-6.
218. Tsai CJ, Aslam K, Drendel HM, et al. Hsp31 Is a Stress Response Chaperone That Intervenes in the Protein Misfolding Process. J Biol Chem 2015;290:24816-34.

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