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RNA Metabolism in the Pathogenesis of Parkinson's Disease

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Abstract

Neurodegenerative diseases such as Parkinson's disease are progressive disorders of the nervous system that affect the function and maintenance of specific neuronal populations. While most disease cases are sporadic with no known cause, a small percentage of disease cases is caused by inherited genetic mutations. The identification of genes associated with the familial forms of the diseases and subsequent studies of proteins encoded by the disease genes in cellular or animals models have offered much-needed insights into the molecular and cellular mechanisms underlying disease pathogenesis. Recent studies of the familial Parkinson's disease genes have emphasized the importance of RNA metabolism, particularly mRNA translation, in the disease process. It is anticipated that continued studies on the role of RNA metabolism in Parkinson's disease will offer unifying mechanisms for understanding the cause of neuronal dysfunction and degeneration and facilitate the development of novel and rational strategies for treating this debilitating disease.

Keywords

Parkinson's disease; mRNA metabolism; Translation initiation; MicroRNAs; Oxidative stress; Mitochondria; mTOR signaling

Introduction

Parkinson's disease (PD) is the most common movement disorder and the second most common neurodegenerative disease. Aging remains one of the most significant risk factors for the disease. Approximately 1% of the population is affected at 65 years of age. This is increased to 4–5% in 85 years and older individuals (Lang and Lozano, 1998a, b). Disease is characterized clinically by motor dysfunction that manifests as resting tremor, bradykinesia, rigidity and postural instability. The movement abnormality in PD is largely attributable to the deficiency of brain dopamine content caused by dysfunction and degeneration of dopaminergic neurons in the substantia nigra. Neurons in other brain areas are also affected, which may contribute to the non-motor symptoms of the disease such as depression, dementia, anxiety, and sleeping disturbance. A pathological hallmark of the disease is the formation of Lewy bodies, intracytoplasmic inclusions that are composed mainly of α -

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synuclein (α -Syn) and ubiquitin, although Lewy body pathology is absent in some disease cases. The most common forms of PD are sporadic with no known cause. Nevertheless, postmortem studies of sporadic cases have revealed protein aggregation, defective mitochondrial respiratory chain function, and oxidative damage of nigrostriatal dopaminergic neurons as shared features of the disease (Dawson and Dawson, 2003; Dexter et al., 1994; Dunnett and Bjorklund, 1999; Schapira and Jenner, 2011).

It is now well established that genetic factors make significant contributions to PD pathogenesis. Through linkage analysis, genome sequencing and genetic association, many genes have been linked to PD (Dawson et al., 2010; Trinh and Farrer, 2013). Understanding the genetic and biochemical relationships among the disease genes and elucidating the signaling pathways underlying disease pathogenesis remain major challenges of the field. In this respect, genetic studies in animal models of PD, or RNAi-based genome-wide screens for modifiers of PD-relevant cellular phenotypes in human cell culture models have the potential to unravel the molecular mechanisms underlying disease pathogenesis and offer novel and rational therapeutic strategies. Although the implied molecular functions of the disease-associated genes are diverse and the cellular processes they are involved in are still poorly understood, recent studies have emphasized the importance of RNA metabolism, especially translational regulation, in either mediating or regulating the function of several disease-causing gene products.

In this review, we will summarize studies linking PD-related genes to the regulation of mRNA translation and the reciprocal role of RNA metabolism in regulating the function of PD-causing genes. We will discuss how altered mRNA translation may contribute to PD pathogenesis and suggest future directions for further investigation.

Translational regulation

Regulated mRNA translation offers one important mechanism of cell growth and survival control. By regulating translation, an organism is able to generate quick responses to physiological or environmental cues by directing the expression of proteins from existing cellular mRNAs. Translational control plays a critical role during early development of most metazoans, because zygotic transcription does not occur during the first few hours of life and cells rely solely on maternal RNAs and proteins (Wilhelm and Smibert, 2005). Under stress conditions in adult animals, translational regulation promotes survival by upregulating the expression of a subset of protective proteins and down-regulating non-essential proteins to conserve energy resources (Holcik and Sonenberg, 2005). Although translation rates can be regulated at each of three steps: initiation, elongation, and termination, control of translation initiation has emerged as a major regulatory mechanism under various pathophysiological conditions.

Increased protein synthesis correlates with enhanced formation of the eIF4F initiation complex, which binds to the 5' cap of eukaryotic mRNAs containing the m⁷GpppX structure (Gingras et al., 1999). eIF4F mediates the binding of mRNAs to the 43S pre-initiation complex to form the 48S initiation complex. eIF4F is a multi-protein complex, with eIF4G, eIF4A, and eIF4E being its known subunits. eIF4G is a scaffolding protein that binds to eIF4E and eIF4A and also interacts with proteins in the 43S pre-initiation complex (Gradi et

al., 1998). eIF4A is an ATP-dependent helicase that unwinds secondary structures present in the 5' untranslated regions (UTRs) of certain mRNAs (Ray et al., 1985). The 5' cap-binding activity of the complex is conferred by the eIF4E subunit (Sonenberg et al., 1979), which appears to be the rate-limiting factor in the formation of eIF4F, and whose activity is tightly regulated under cell growth conditions. The activity of eIF4E is regulated by the binding of eIF4E by 4E-BP, which precludes the binding of eIF4E by eIF4G and eIF4A, thus preventing the formation of the eIF4F complex (Gingras et al., 1999; Richter and Sonenberg, 2005). 4E-BP has been established as a major downstream effector of the target of rapamycin (TOR) signalling pathway, which tightly couples nutrient availability, growth factors, and energy status to cell growth (Wullschleger et al., 2006). TOR forms at least two protein complexes, TORC1 and TORC2 (Zoncu et al., 2011). TORC1 has been shown to phosphorylate 4E-BP, weakening its binding to eIF4E and promoting cap-dependent mRNA translation. In addition, TORC1 stimulates protein synthesis by positively regulating the activity of ribosomal protein S6 kinase (S6K) through direct phosphorylation (Zoncu et al., 2011). Various stress signals have been shown to influence mRNA translation and metabolism by impinging on the TOR pathway (Wullschleger et al., 2006). Intriguingly, several proteins encoded by PD-associated genes have recently been shown to interact with components of the translation initiation complex or signaling pathways that regulation mRNA translation (Figure 1).

Leucine rich repeat kinase 2 (LRRK2) (OMIM #609007)

LRRK2 encodes a large protein that contains a leucine rich repeat (LRR) domain, a kinase domain, a RAS-like GTPase domain, and a WD-40 domain (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). Mutations in *LRRK2* are the most frequently found genetic lesions associated with familial as well as sporadic PD cases, indicating a general role for *LRRK2* dysfunction in disease pathogenesis (Taylor et al., 2006). To understand *LRRK2* pathogenesis, animal models recapitulating key features of disease have been established in different species. For example, expression of the p.I2020T mutant form of *Drosophila* LRRK (dLRRK) or the p.G2019S mutant forms of hLRRK2 resulted in rather selective dopaminergic (DA) neuron degeneration in *Drosophila* (Imai et al., 2008; Liu et al., 2008; Ng et al., 2009; Venderova et al., 2009). Mouse *LRRK2* models displayed an age-dependent and levodopa-responsive slowness of movement associated with diminished dopamine release and axonal pathology of nigrostriatal dopaminergic projection in mutant *LRRK2*(R1441G) BAC transgenic mice (Li et al., 2009), or progressive degeneration of substantia nigra *pars compacta* (SNpc) DA neurons and parkinsonism phenotypes of motor dysfunction in *LRRK2* (G2019S) transgenic mice (Chen et al., 2012).

Imai et al found that both human *LRRK2* and dLRRK phosphorylate 4E-BP, a negative regulator of eIF4E-mediated protein translation and a key mediator of cellular response to various stress conditions in *Drosophila*, such as bacterial infection, starvation, and oxidative stress (Imai et al., 2008). Modulation of the eIF4E/4E-BP pathway by certain pathogenic forms of *LRRK2* stimulates eIF4E-mediated translation initiation, resulting in attenuated resistance to oxidative stress and compromised survival of DA neuron in *Drosophila*. This result suggested that chronic inactivation of 4E-BP by mutant *LRRK2* carrying certain pathogenic mutations, such as the most common p.G2019S mutation, deregulates mRNA

translation and cause age-dependent loss of DA neurons (Imai et al., 2008). This study for the first time directly implicates deregulated mRNA translation initiation in PD pathogenesis. Studies of LRRK2 effect on 4E-BP phosphorylation in mammalian cells and tissues have generated a somewhat more complicated picture. While some studies have confirmed LRRK2 phosphorylation of 4E-BP *in vitro* (Kumar et al., 2010; Trancikova et al., 2012) but found little change of 4E-BP phosphorylation *in vivo* in LRRK2 loss- or gain-of-function conditions (Trancikova et al., 2012), other have found increased 4E-BP phosphorylation in LRRK2 knockout (KO) and LRRK2 kinase dead (KD) transgenic mice but not in LRRK2(G2019S) knock-in (KI) mice (Herzig et al., 2011). Thus, the exact roles of WT and pathogenic LRRK2 proteins on 4E-BP phosphorylation and function in mammals await further investigation.

Although the eIF4E/4E-BP pathway is considered to be generally involved in translational control, there is evidence that only a small subset of total cellular mRNAs is translationally regulated by this pathway (Holcik and Sonenberg, 2005). To identify the cellular mRNA targets whose translation is affected by pathogenic LRRK2 through the eIF4E/4E-BP pathway, Gehrke *et al* used a translational mRNA profiling method to identify *e2f1* and *dp* mRNAs as cellular mRNAs that are translationally derepressed by pathogenic LRRK2 (Gehrke et al., 2010). E2F1 and DP are key regulators of cell cycle progression in proliferating cells. However, their activation in postmitotic neurons can induce cell death and this contributes to the dopaminergic neurodegeneration effect caused by pathogenic LRRK2. Intriguingly, Gehrke *et al* revealed that a major part of the effect of LRRK2 on translational regulation is through the miRNA pathway, which is known to modulate mRNA translation or stability. They further identified miR-184* and let-7 miRNAs as translational repressors of *Drosophila e2f1* and *dp* messenger RNAs, respectively. Mechanistically, it was shown that pathogenic LRRK2 acts through two modes: 1) it associates with *Drosophila* Argonaute-1 (dAgo1) or human Argonaute-2 (hAgo2) of the RNA-induced silencing complex (RISC), and negatively regulates dAgo1 protein level in aged fly brain through an unknown mechanism; 2) pathogenic LRRK2 promotes the association of phospho-4E-BP1 with hAgo2, resulting in altered hAgo2 function. Interestingly, upregulation of E2F has been suggested to mediate cell death of dopaminergic neurons in patients with Parkinson's disease and in vertebrate models of the disease (Hoglinger et al., 2007). It would be interesting to test whether altered miRNA function is a major cause of E2F deregulation in mammals. A recent study suggested that the expression of human LRRK2 is repressed by the action of microRNA-205 (miR-205) through a conserved binding site at the 3'-untranslated region (UTR) of *LRRK2* gene, and that miR-205 expression was significantly downregulated in the brains of patients with sporadic PD, which exhibited enhanced LRRK2 protein levels (Cho et al., 2013). It would be interesting to test whether the expression and/or function of miR-205 is affected by pathogenic LRRK2 and whether there exists a feed-forward regulatory loop that leads to the upregulation of LRRK2 protein levels in sporadic PD cases. These studies also raised the possibility of miRNA-based biomarker identification (Khoo et al., 2012) and therapeutic (Mouradian, 2012) strategies for PD.

Although the functional studies support the critical role of deregulated *e2f1* and *dp* mRNA translation by the eIF4E/4E-BP pathway in LRRK2 pathogenesis, this does not exclude the

involvement of other deregulated cellular mRNAs in the disease process. A recent study of the transcriptome regulated by 4E-BP revealed a surprisingly large number of mRNAs, many encoding mitochondrial respiratory chain complex (RCC) subunits, whose translation is unexpectedly positively controlled by 4E-BP (Zid et al., 2009). The authors explored the role of translational regulation in the context of lifespan extension by dietary restriction, an environmental manipulation that extends lifespan across species. They found that under dietary restriction conditions, 4E-BP is upregulated, resulting in increased ribosomal loading of nuclear-encoded mitochondrial *RCC* mRNAs, enhanced overall mitochondrial activity, and lifespan extension. A key feature shared by the various mitochondrial *RCC* genes is that they possess shorter and less structured 5'UTRs important for their enhanced mRNA translation. This study implicates translational regulation of nuclear-encoded mitochondrial *RCC* gene expression by 4E-BP in influencing mitochondrial activity and organismal lifespan. It would be interesting to understand the molecular mechanism by which 4E-BP positively regulates the translation of mitochondrial *RCC* mRNAs. It would also be interesting to test whether this critical mitochondrial regulation by 4E-BP is altered by pathogenic *LRRK2*, which is known to cause mitochondrial dysfunction (Cooper et al., 2012), a cardinal feature of Parkinson's disease.

Parkin (OMIM #602544) and Pten-induced kinase 1 (PINK1, OMIM#608309)

Parkin gene encodes a ring finger domain-containing E3 ubiquitin ligase. Its inactivation causes autosomal recessive juvenile parkinsonism (Kitada et al., 1998). To understand the mechanism by which *Parkin* inactivation leads to parkinsonism, various animal models have been established. Three labs independently generated *parkin* null mutant flies (Cha et al., 2005; Greene et al., 2003; Pesah et al., 2004). A prominent feature of *parkin* mutant flies is their mitochondrial pathology and apoptotic muscle degeneration. In addition, whole mount confocal analysis detected subtle but consistent dopaminergic neuron loss in the protocerebral posterior lateral (PPL) 1 cluster in *parkin* null flies (Whitworth et al., 2005). *parkin* mutant flies also exhibit sterility, reduced lifespan, reduced cell number and size, and hypersensitivity to oxidative stress. Using a different approach, *Parkin* function was inhibited by transgenic RNAi (Yang et al., 2003). *Parkin* RNAi flies exhibited genetic interaction with transgenic flies overexpressing human Pael-R, a substrate protein ubiquitinated by *Parkin*'s E3 ligase activity (Imai et al., 2001), consistent with Pael-R being a *Parkin* substrate that mediates *Parkin*-associated parkinsonism. Recent studies in mice also showed that Pael-R induces dopaminergic neurons degeneration in the substantia nigra, and that Pael-R toxicity is enhanced under *Parkin* inactivation condition (Kitao et al., 2007). Surprisingly, overexpression of certain mutant forms of human *Parkin* in *Drosophila* also led to dopaminergic neurodegeneration and mitochondrial abnormality in indirect flight muscle (Sang et al., 2007; Wang et al., 2007), suggesting that at least some of the FPD mutations in *Parkin* may have gained certain toxic property or act in a dominant-negative fashion. Analyses of *parkin* KO mice have emphasized the importance of *Parkin* in regulating evoked dopamine release and striatal synaptic plasticity in the nigrostriatal pathway (Goldberg et al., 2003). Interestingly, although *parkin* $-/-$ mice generated by conventional KO did not show obvious dopaminergic degeneration (Goldberg et al., 2003), conditional *parkin* KO in adult animals led to progressive loss of DA neurons (Shin et al.,

2011), suggesting that certain compensatory responses elicited during early development might have masked *parkin* mutant phenotypes in the conventional knockout animals.

PINK1 encodes a Ser/Thr kinase with an N-terminal mitochondrial targeting domain (Unoki and Nakamura, 2001). Its loss of function results in early onset, autosomal-recessive parkinsonism (Valente et al., 2004). The identification of *PINK1* as a PD-causing gene offers the strongest genetic evidence that mitochondrial dysfunction is at the center of PD pathogenesis. Inhibition of *Drosophila PINK1* (*dPINK1*) resulted in phenotypes strikingly similar to that seen in *parkin* mutant flies, including male sterility, apoptotic muscle degeneration, dopaminergic neuron loss, defective mitochondrial morphology, and increased sensitivity to multiple stresses including oxidative stress (Clark et al., 2006; Park et al., 2006; Wang et al., 2006; Yang et al., 2006). It is intriguing that the indirect flight muscle and DA neurons are particularly vulnerable to *PINK1* and *parkin* mutations. It is possible that high endogenous levels of oxidative stress in these tissues and cell types may confer this selective vulnerability. Being one of the most metabolically active tissue, fly indirect flight muscle probably produces more ROS than any other tissues and is presumably under constant oxidative stress (Sacktor, 1976). Dopaminergic neurons are also constantly under oxidative stress due to the oxidative metabolism of dopamine. Together, these conditions may confer particular vulnerability of these tissues or cell types to additional oxidative insults caused by mitochondrial dysfunction in *PINK1* or *parkin* mutants.

The similar phenotypes observed in *parkin* and *PINK1* mutant flies prompted genetic epistasis studies to investigate their relationships. Overexpression of Parkin largely rescued the *PINK1* mutant phenotypes, whereas overexpression of *PINK1* had no effect on *parkin* mutant phenotypes. Further, *PINK1 parkin* double mutants showed phenotypes identical to those observed in single mutants. These observations suggest that *PINK1* and Parkin function in the same pathway, with *PINK1* acting upstream of Parkin (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). Studies in mammals have largely confirmed the conservation of the functional relationship between *PINK1* and Parkin in regulating mitochondrial morphology and function (Exner et al., 2007; Narendra et al., 2010).

Genetic studies in *Drosophila* have further implicated the *PINK1*/Parkin pathway in regulating mitochondrial morphology through interaction with the mitochondrial fission and fusion pathway genes (Deng et al., 2008; Park et al., 2009; Poole et al., 2008; Yang et al., 2008). Mitochondrial fission and fusion are evolutionarily conserved membrane remodeling processes that regulate the morphology and subcellular distribution of mitochondrial units (Chan, 2006). Genetic studies in *Drosophila* strongly suggested that the mitochondrial morphology defects in *PINK1* and *parkin* mutants are due to decreased fission or increased fusion. Two dynamin-like GTPases, Drp1 and Opa1, are critical components of the machinery that regulate mitochondrial fission and fusion, respectively. Enhancing mitochondrial fission activity by the overexpression of Drp1 or reducing fusion activity by the loss of one copy of *Opa1* can rescue the DA neuron loss defect in *Pink1* mutant (Yang et al., 2008). The biochemical mechanisms linking *PINK1*/Parkin to mitochondrial fission/fusion machinery remain to be determined. Parkin may directly ubiquitinate and promote the degradation of the mitochondrial fusion regulator Mfn (or Marf in *Drosophila*), thereby inhibiting mitochondrial fusion (Ziviani et al., 2010), or it may positively regulate the fission

regulators Drp1 and Fis1. Interestingly, a recent study implicated the valosin-containing protein (VCP) gene in the regulation of mitochondrial dynamics by the PINK1/Parkin pathway (Kim et al., 2013). VCP interacts genetically with the PINK1/parkin pathway *in vivo*, and VCP recruitment to damaged mitochondria requires Parkin-mediated ubiquitination of mitochondrial targets. Interestingly, VCP recruitment coincides temporally with mitochondrial fission, and VCP is required for the proteasome-dependent degradation of Mfns *in vitro* and *in vivo*. The implication of PINK1/Parkin in promoting fission or inhibiting fusion is consistent with the notion that the PINK1/Parkin pathway plays a central role in mitochondrial quality control (Youle and Narendra, 2011), during which PINK1 becomes stabilized on damaged mitochondria, recruits Parkin from the cytosol, and targets damaged mitochondria for clearance by autophagy (mitophagy). The pro-fission or anti-fusion role of PINK1/Parkin would ensure that badly damaged mitochondria be culled from the healthy mitochondrial network (Twig et al., 2008). However, it is worth noting that the majority of the studies demonstrating the role of the PINK1/Parkin pathway in mitophagy so far relied upon the application of the mitochondrial membrane potential uncoupler CCCP to cells. The mechanisms of action of PINK1 and Parkin in maintaining mitochondrial health under normal physiological conditions remain to be elucidated.

In a genetic screen for modifiers of *PINK1* in *Drosophila*, it was found that inhibition of TORC1 signaling effectively suppressed *PINK1* mutant phenotypes (Liu and Lu, 2010). The effect of reduced TORC1 signaling on *PINK1* mutant phenotypes is mediated largely through the regulation of mRNA translation, since inhibition of S6K or overexpression of 4E-BP had similar effect, whereas S6K overexpression had opposite effects (Liu and Lu, 2010). Interestingly, TORC1 signaling was downregulated when PINK1 is inhibited, presumably reflecting a protective, compensatory cellular response. This study demonstrated that downregulation of global mRNA translation is beneficial in *PINK1* mutant condition. Since mRNA translation is highly energy demanding, downregulation of global mRNA translation may rescue *PINK1* mutant phenotypes by conserving the limited energy supply in these mutants. Intriguingly, the enhanced *PINK1* mutant phenotype in the presence of activated S6K could be fully rescued by Parkin, apparently in an autophagy-independent manner. The protective effect of 4E-BP overexpression against *PINK1* mutation was also reported in another study (Tain et al., 2009). These authors further showed that the TOR inhibitor rapamycin had similar effect as 4E-BP overexpression in suppressing pathology in *PINK1* and *Parkin* mutants, and that the effect of rapamycin is mediated mainly through mRNA translation rather than autophagy (Tain et al., 2009). Together, these studies demonstrated the importance of altered mRNA translational regulation in PD pathogenesis. Paradoxically, although both TORC1 and TORC2 signaling are downregulated in *PINK1* mutant condition, unlike in TORC1 signaling case, restoration of TORC2 function is beneficial, whereas further inhibition of TORC2 signaling is detrimental (Wu et al., 2013). It would be interesting to explore the molecular mechanisms mediating the differential effects of TORC1 and TORC2 signaling in PINK1/Parkin-related PD pathogenesis.

Whether PINK1/Parkin may play a more direct role in regulating mRNA translation remains to be established. Recent studies of the interactome of Parkin in mammalian cells have identified a number of translation initiation-related regulatory factors and ribosomal proteins

as potential Parkin-interacting proteins (Sarraf et al., 2013; Zanon et al., 2013). Although these protein-protein interactions remain to be validated and their functional roles remain uncertain, these results nevertheless suggest a more direct role of Parkin in mRNA translation. This would be consistent with earlier studies identifying p38, a key structural component of the mammalian aminoacyl-tRNA synthetase complex involved in protein synthesis, as a substrate of Parkin-mediated ubiquitination (Corti et al., 2003; Ko et al., 2005). The potential role of PINK1 in directly regulating mRNA translation is less clear. In *Drosophila*, PINK1 was predicted based on yeast two-hybrid study to interact with the *Drosophila* homologue of the mammalian mitochondrial translation initiation factor 3 (MTIF3), which promotes the initiation complex formation on 55S mitochondrial ribosomes. Intriguingly, in two independent case-control collections, a c.798C>T polymorphism of the *MTIF3* gene showed allelic association with PD (Abahuni et al., 2007). Although this association requires further validation in larger studies of patients from various ethnic groups, it raised the possibility that altered translation of mitochondrial-encoded mRNAs can lead to susceptibility to PD.

A recent study in mammalian cells identified a novel splice variant of *PINK1* (*svPINK1*) mRNA that encodes the C-terminus regulatory domain of the protein kinase, and that a noncoding antisense RNA expressed at the *PINK1* locus (*naPINK1*) showed concordant regulation with *svPINK1* during *in vivo* conditions requiring mitochondrial biogenesis (Scheele et al., 2007). Selective targeting of *naPINK1* by RNAi resulted in loss of *svPINK1* in neuronal cell lines. Thus, *naPINK1* can positively influence the abundance of *cis*-transcribed *svPINK1* mRNA under physiological conditions, presumably through a dsRNA-mediated mechanism. This result reveals the complexity of the genomic regulation of the *PINK1* locus, and a role of RNA metabolism in regulating PINK1 expression. Given the prevalence of long non-coding RNAs (lncRNAs) and repetitive sequences in the mammalian genome, one can assume that there will be many examples of translational regulation of important disease-relevant genes by lncRNAs. A recent study identified a nuclear-enriched lncRNA antisense to *ubiquitin carboxy-terminal hydrolase L1* (*Uchl1*), a gene linked to rare familial PD. Interestingly, *Uchl1* lncRNA also increases UCHL1 protein synthesis at a post-transcriptional level, apparently by promoting the association of the overlapping protein-coding *Uchl1* mRNA to active polysomes. Moreover, *Uchl1* lncRNA function is regulated by stress signalling pathways. For example, TORC1 inhibition by rapamycin causes the shuttling of *Uchl1* lncRNA from the nucleus to the cytoplasm (Carrieri et al., 2012). It would be interesting to test the generality of lncRNA regulation by mTOR signaling for other disease-associated genes such as PINK1 and its role in disease pathogenesis.

Eukaryotic translation initiation factor 4-gamma (EIF4G1, OMIM #600495)

Another link between translational initiation and PD pathogenesis was provided by the identification of mutations in *EIF4G1* in rare familial cases of PD (Chartier-Harlin et al., 2011). Genome-wide analysis of a multi-incident family with autosomal-dominant parkinsonism implicated a locus on chromosomal region 3q26-q28. Missense mutations in *EIF4G1* gene located in this region were found in affected subjects with familial parkinsonism and idiopathic Lewy body disease but not in control subjects. Biochemically, pathogenic *EIF4G1* mutations were found to disrupt eIF4E or eIF3E binding, rendering

mutant cells more vulnerable to oxidative stress (Chartier-Harlin et al., 2011). The identification of *EIF4G1* mutations associated to PD reinforces the notion that defective mRNA translational initiation can lead to parkinsonism (Imai et al., 2008). eIF4G harbors aggregation-promoting prion-like domains commonly found in RNA-binding proteins, which allow them to rapidly self-associate and form ribonucleoprotein granules such as stress granules (SGs) and processing (P)-bodies (King et al., 2012). SGs and P-bodies are sites where nontranslating mRNAs as well as factors involved in translation regulation and mRNA decay are preferentially distributed when translation is stalled or impeded under unfavorable conditions. In fact, eIF4G and other translation initiation-related factors were found in SGs (Kedersha et al., 2002). It would be interesting to test the potential role of SGs and P-bodies in eIF4G- and mRNA translation-related PD pathogenesis. It should be noted that since the first report of the association of eIF4G mutations to familial PD, unaffected carriers of such variants have been found (Lesage et al., 2012). This discrepancy could be age-associated or due to incomplete penetrance of the mutations, or that the original linkage assignment could be in error. Further studies are needed to support the pathogenic role of *eIF4G1* mutations in PD.

DJ-1 (OMIM #602533)

DJ-1 encodes a highly conserved protein belonging to the ThiJ/PfpI superfamily (Nagakubo et al., 1997). Like PINK1 and Parkin, recessive loss of function in *DJ-1* resulted in parkinsonism (Bonifati et al., 2003). The proposed molecular functions of DJ-1 include molecular chaperone, RNA binding, and anti-oxidant. There are two DJ-1 homologues in *Drosophila*, named DJ-1A and DJ-1B. DJ-1A appears to be a closer homologue of human DJ-1 than DJ-1B based on sequence conservation. DJ-1A and DJ-1B knockout flies display altered sensitivity to environmental toxins such as paraquat, H₂O₂, and rotenone (Menzies et al., 2005; Meulener et al., 2005). Knock down of DJ-1A by transgenic RNAi in a cell type-specific fashion resulted in accumulation of reactive oxygen species, hypersensitivity to oxidative stress, and dysfunction and degeneration of dopaminergic and photoreceptor neurons (Yang et al., 2005). DJ-1 knockout mice were created to study the normal function of DJ-1 in mammals (Goldberg et al., 2005). These mice had normal numbers of DA neurons in the substantia nigra, suggesting that DJ-1 may not be essential for the maintenance of these disease-relevant neurons in mouse. However, evoked dopamine overflow in the striatum was markedly reduced, primarily as a result of increased reuptake. Moreover, nigral neurons lacking DJ-1 were less sensitive to the inhibitory effects of D2 autoreceptor stimulation, and corticostriatal long-term depression (LTD) was absent in medium spiny neurons, although LTP was normal, suggesting a critical role for DJ-1 in dopaminergic physiology (Goldberg et al., 2005).

To understand the molecular mechanisms underlying DJ-1 function *in vivo*, a genetic screen for modifiers of DJ-1A RNAi-induced cell death was carried out in *Drosophila*. This led to the isolation of components of the PI3K/Akt signaling pathway as specific genetic modifiers (Yang et al., 2005). An independent study also found DJ-1A as a genetic modifier of PTEN-induced small-eye phenotype in *Drosophila*, and that DJ-1 RNAi or overexpression alters the phosphorylation of PKB/Akt (Kim et al., 2005). Interestingly, in primary breast cancer samples, DJ-1 expression correlates negatively with PTEN immunoreactivity and positively

with Akt hyperphosphorylation (Kim et al., 2005). DJ-1 thus appears to be a key negative regulator of PTEN, and may serve as a useful prognostic marker for cancer. These initial studies raised the interesting possibility that cancer and Parkinson's disease, two seemingly disparate diseases, may share certain underlying biochemical pathways. Indeed, this has turned out to be a recurring theme in later studies of PD-associated genes (Devine et al., 2011).

The specific molecular function of DJ-1 relevant to PD pathogenesis remains unknown. Given that the molecular marker used to measure Akt phosphorylation in the DJ-1 models actually reflects Akt phosphorylation by TORC2 (Kim et al., 2005; Sarbassov et al., 2005; Yang et al., 2005), it is possible that similar to the situation in *PINK1* mutant, TOR signaling is compromised in *DJ-1* mutant, suggesting a common molecular pathway underlying recessive parkinsonism. Consistent with this notion, *DJ-1* has been shown to genetically interact with *PINK1* in *Drosophila* models (Hao et al., 2010). One study showed that DJ-1 binds to mRNA targets in cells and brain tissues in an oxidation-dependent manner. The RNA targets of DJ-1 include mitochondrial genes, genes involved in glutathione metabolism, and members of the PTEN/PI3K cascade (van der Brug et al., 2008). It would be interesting to determine the mode of RNA-binding by DJ-1 and to test whether DJ-1 may regulate the translation of these mRNA targets or their subcellular localization under stress conditions. It would also be interesting to test the interaction and cooperation between DJ-1 and PINK1/Parkin in regulating mRNA metabolism.

α -Synuclein (α -Syn, OMIM #163890)

α -Syn encodes a small protein whose normal function is likely related to synaptic transmission (Maroteaux et al., 1988). It is a main component of Lewy bodies and its aggregation is frequently found in PD (Spillantini et al., 1997). Missense mutations or genomic triplication of the α -Syn gene have been linked to autosomal dominant familial PD (Polymeropoulos et al., 1997; Singleton et al., 2003). To elucidate the pathogenic role of α -Syn in PD pathogenesis, transgenic mice expressing wild-type human α -Syn were generated (Masliah et al., 2000). Neuronal expression of human α -Syn resulted in progressive accumulation of α -Syn and ubiquitin-immunoreactive inclusions, manifested as both electron-dense intranuclear deposits and cytoplasmic inclusions. These alterations were associated with loss of dopaminergic terminals in the basal ganglia and with motor impairments, suggesting that accumulation of wild-type α -Syn can play a causal role in PD pathogenesis (Masliah et al., 2000). Although *Drosophila* does not have a clear α -Syn homologue, overexpression of wild-type and FPD-associated mutant human α -Syn in *Drosophila* has recapitulated key features of PD, including Lewy body-like aggregate formation, selective degeneration of dopaminergic neurons, and locomotor behavior abnormality (Auluck et al., 2002; Feany and Bender, 2000). The fact that both mutant and wild-type α -Syn can form aggregates in fly neurons and induce disease phenotypes supports the notion that improper disposal of aggregation-prone abnormal proteins can result in neurodegeneration. This is also consistent with the finding that increased α -Syn protein production by genomic triplication of endogenous α -Syn gene can cause PD (Singleton et al., 2003). Consistent with a role for aberrant protein quality control in α -Syn pathogenesis, the molecular chaperone HSP70 has been shown to modulate α -Syn pathogenesis in

Drosophila (Auluck et al., 2002) or mice (Dong et al., 2005), and polymorphisms in the *Hsp70* genes may confer susceptibility to PD in humans (Wu et al., 2004).

Despite these studies, the exact cellular mechanism by which accumulation of wild type α -Syn or expression of mutant α -Syn causes selective DA neuron degeneration remains elusive. Systematic genetic studies in model organisms promise to address this question. Studies initially carried out in yeast showed that α -Syn can cause a block in endoplasmic reticulum (ER)–to–Golgi vesicular trafficking. A genome-wide genetic screen identified toxicity modifiers, including the Rab guanosine triphosphatase Ypt1p, which associated with cytoplasmic α -Syn inclusions. Elevated expression of Rab1, the metazoan *YPT1* homolog, protected against α -Syn-induced DA neuron loss in PD models (Cooper et al., 2006). This study revealed basic cellular functions disrupted by toxic α -Syn expression that may contribute to synucleinopathies. Recent studies using patient-derived induced pluripotent stem cell (iPSC)-based neuronal models of PD have validated the conservation of this cellular mechanism in α -Syn pathogenesis (Chung et al., 2013).

Given that the expression level of α -Syn protein can significantly influence neuronal physiology and survival, and that elevated α -Syn protein expression is found in familial and sporadic PD, control of α -Syn protein expression presents a key regulatory mechanism. The human α -Syn mRNA has a long 3'-UTR that is highly conserved (Sotiriou et al., 2009), suggesting potential functional significance of the 3'-UTR in regulating α -Syn mRNA stability and/or translation. Recent reports demonstrate that the α -Syn mRNA is negatively regulated by microRNAs (Mouradian, 2012). Repression of α -Syn protein expression by miR-7 and miR-153 microRNAs protects against the impaired proteasomal function and cytotoxicity associated with α -Syn over-expression in cultured cells (Doxakis, 2010; Junn et al., 2009). Moreover, microRNA profiling approaches have uncovered variations in the abundance of certain microRNAs that may be relevant to disease pathogenesis (Martins et al., 2011). For example, miR-34b/34c are decreased in several affected brain regions in PD and Lewy body disease. However, no significant changes in miR-7 or miR-153 levels have been observed. Similarly, polymorphisms in the 3'-UTR region of human α -Syn gene have been identified, but none corresponds to the binding sites for miR-7 or miR-153 miRNAs. Thus, if altered functions of miR-7 and miR-153 miRNAs contribute to PD pathogenesis, their effect may depend on cooperation with trans-acting protein factors or other miRNAs that also act on the conserved 3'-UTR region of human α -Syn mRNA to regulate its stability, localization, or translation. A recent study suggest that specific α -Syn mRNA isoforms with an extended 33UTR (α -Syn L) are selectively linked to pathological processes (Rhinn et al., 2012). The authors showed that common variants in α -Syn 33UTR associated with PD risk promote the accumulation and translation of α -Syn L transcripts. Intriguingly, the presence of intracellular dopamine can further enhance the relative abundance of α -Syn L mRNAs through alternative selection of polyadenylation (polyA) sites. Moreover, the presence of the extended α -Syn L 33UTR impacts the accumulation of α -Syn protein, which appears to be redirected towards mitochondria and away from synaptic terminals. These results reveal unanticipated complexity of α -Syn regulation by RNA metabolism in the context of PD-associated genetic and environmental changes.

How might RNA metabolism impact PD pathogenesis?

Here we propose a number of scenarios whereby altered RNA metabolism may impact PD pathogenesis. We stress that these mechanisms are not mutually exclusive. In fact, we envision that these mechanisms may act in concert in the course of the disease.

1. Reprogramming of the proteome under stress conditions. Cells constantly encounter stressful challenges from internal and external sources. Being able to quickly adapt to these challenges is essential for maintaining cell function and ensuring survival. Of particular relevance to PD, DA neurons may face elevated oxidative stress caused by dopamine metabolism and energy depletion caused by their unique electrophysiology. Adaptive RNA metabolism allows cells to quickly increase the synthesis of proteins important for survival (e.g., anti-oxidants, mitochondrial proteins, chaperones, anti-apoptotic proteins, etc) and reduce the synthesis of non-essential proteins.
2. Conservation of energy. Given that protein synthesis is a very energy-consuming process, consuming approximately 80% of the energy in proliferating cells, reduction of global protein synthesis may help conserve energy under stress conditions. This is especially important under disease conditions where mitochondrial dysfunction is causal, such as PINK1-associated PD. This may explain the rescuing effect by measures that reduce protein synthesis, such as TOR inhibition or 4E-BP overexpression, in multiple PD models.
3. Modulating the levels of misfolded or aggregated proteins. Altered RNA metabolism, such as reduction of mRNA levels or rate of mRNA translation by microRNAs, offers cells the ability to better manage the levels of misfolded or aggregation-prone proteins such as α -Syn, the accumulation of which can be directly causal of PD.
4. Distribution of mRNAs among different intracellular ribonucleoprotein granules. mRNAs are present in various large ribonucleoprotein granules *in vivo*. For example, neurons contain transport granules in which mRNAs are translationally repressed while being distributed to neuronal synapses and other subneuronal locations. Under stress, translationally stalled mRNAs are degraded in P-bodies or stored in stress granules. Being able to maintain the dynamic equilibrium of mRNA distribution among the various intracellular ribonucleoprotein granules is likely to be particularly important to neuronal function, as a number of neurological disorders are caused by RNA-binding proteins involved in ribonucleoprotein granule formation or function. We envision that altered RNA metabolism leading to defective ribonucleoprotein granule formation or function may play a much broader role in the pathogenesis of neurodegenerative diseases including PD and present a fertile ground for future exploration.

Conclusion

Genetic and biochemical studies of familial PD-associated genes have strongly implicated altered RNA metabolism in PD pathogenesis. In particular, several familial PD genes,

including *LRRK2*, *PINK1*, *Parkin*, and *eIF4G1*, have been shown to interact with components of the translation initiation machinery or interact with modulators of the translation initiation process, such as miRNAs and the mTORC1 signaling pathway. Future studies aimed at elucidating the detailed molecular mechanisms of translational regulation by familial PD genes and how modulation of translational initiation, a seemingly ubiquitously important cellular process, can result in selective DA neuron degeneration, will deepen our understanding of PD pathogenesis. Further studies of RNA metabolism in PD pathogenesis will also offer novel biomarkers of PD. It is therefore hopeful that continued studies on the role of RNA metabolism in PD will advance our understanding of the cause of neuronal dysfunction and degeneration and facilitate the development of novel and rational strategies for the early diagnosis and treatment of this debilitating disease.

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Figure 1. A diagram of a signaling network regulating translation initiation that has been linked to PD-pathogenesis caused by mutations in LRRK2 or PINK1/Parkin

LRRK2 has been shown to negative regulate the stability of Ago, a component of the Risc/ miRNA repressor complex that inhibits translation initiation. LRRK2 has also been shown to phosphorylate 4E-BP, releasing the repression of eIF4E/eIF4G-mediated translation initiation by 4E-BP, similar to the regulation of 4E-BP by mTORC1. The relationship between PINK1/Parkin and LRRK2 is based on the results of genetic epistasis studies carried out in *Drosophila* and *C. elegans*, although the precise biochemical mechanism underlying the genetic interactions remains to be established.