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Targeting mRNA translation in Parkinson's disease

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders. The exact cause(s) of PD is not well understood, although genetic mutations are associated with some forms of the disease. Many of these mutations, in particular those that are found in *LRRK2*, *DJ-1*, *PINK1*, and *Parkin*, are linked to the deregulation of mRNA translation, suggesting that this process is important for the onset of PD. Herein, we highlight recent studies that provide insights into the molecular mechanisms that relate mRNA translation to PD. These studies confirm the central role of translation in PD, emphasising the potential of restoring mRNA translation functionality as a new therapeutic treatment against PD, and providing novel targets for developing new chemical agents to target this disease.

Introduction

PD is one of the most common neurodegenerative disorders [1–4]. It is estimated that 1 in 800 people worldwide has PD [5]. The number of new cases increases with age for both males and females, with peaks between the ages of 70 and 79 [6]. Within the next 12 years, the number of affected people over 50 years of age in the most populous countries is predicted to double compared with 2005 [7]. Numerous studies have been carried out to understand the causes of PD and its potential treatments. Unfortunately, there has been no significant breakthrough since the discovery of levodopa during the 1960s, with levodopa still representing the only compound for palliative treatment of this disease.

PD is characterised by the alteration of function of neuronal cells and their consequent death, which leads to a loss of dopamine, a neurotransmitter that is responsible for the coordination of body movements. The exact cause(s) of the disease is unclear. However, misfolding, aggregation, and accumulation of proteins are considered the most common causes of several neurodegenerative diseases that share similar pathological mechanisms [8,9]. In PD, the aggregation of lipids and proteins, in particular α-synuclein, which leads to aggregates called Lewy bodies, is considered the hallmark of the disease [5,10–12].

The formation of protein aggregates can be a consequence of dysfunctions in protein synthesis and its regulation. The translation of the genetic code into proteins is a complex process, involving multiple cellular components, including the ribosome, translation factors, and RNAs. Mechanistically, the overall translational process can be divided in four steps: initiation, elongation, termination, and ribosome recycling [13]. These steps are tightly regulated to ensure that the correct operation occurs [14]. Through ageing, parts of the translation process can lose their regulation, thus leading to an alteration of protein homeostasis [15]. In PD, mutations in several genes that encode cellular factors involved in translation and its regulation are believed to be contributing factors to the onset of the disease. The characterisation of these gene products could offer potential clinical applications for PD.

In this review, we present recent discoveries about the effect of deregulated mRNA translation in PD, with a particular focus on the products of the genes that are involved in mRNA translation. We highlight the potential of targeting these gene products for the development of new treatments for PD. We hope that this review will encourage further research into understanding the relationship between PD and deregulated mRNA translation, as well as in renewed drug discovery efforts against this disease.

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Potential targetable pathways

LRRK2

Genetic mutations in the gene *LRRK2* are some of the most common causes of familial PD [16,17]. *LRRK2* encodes leucine-rich repeat kinase 2 (LRRK2), which is a large protein of ~280 kDa that comprises multiple domains, including a GTPase domain and one that exhibits kinase activity [18]. These two domains are functionally connected, with the GTPase domain acting as a regulator of the kinase domain. Furthermore, GTP binding is important to maintain the structural stability, and ensure the correct dimerisation, of the LRRK2 protein [19,20]. The exact substrates of LRRK2 are not well understood. Bioinformatic studies and *in vitro* kinetic assays suggested that LRRK2 has a vast and intricate interactome. In particular, LRRK2 was shown to be able to catalyse the phosphorylation of a large number of proteins from various cellular pathways *in vitro* [21]. These include (but are not limited to) proteins that are involved in the cell cycle, apoptosis, autophagy, vesicular trafficking, and immune response. However, despite the large number of *in vitro* substrates, only a few proteins have been (partially) validated as physiologically relevant substrates of LRRK2 *in vivo*. These include ribosomal protein s15 and mitochondrial antioxidant enzyme PRDX3, as well as Rab GTPases, which were identified in a proteomic-based study with mouse PD models [22]. The considerable number of putative LRRK2 substrates versus the exiguous number of *bona fide* LRRK2-interacting proteins suggest that the exact cellular function(s) of LRRK2 are still not clear. In PD, most mutations are present in the kinase domain of LRRK2. The most common mutation causes a glycine-to-serine substitution at position 2019 (G2019S) of LRRK2, which leads to a pathological increase in its enzymatic activity [23–25]. This gain-of-function mutation affects several mechanisms, including mRNA translation and protein homeostasis (Figure 1).

Current PD therapeutic strategies focus on the development of chemical inhibitors that target the increased kinase activity of mutant LRRK2 [26,27]. This strategy has shown to be promising, and LRRK2 kinase inhibitors that are potent, selective, and can penetrate the blood–brain barrier (BBB) have been reported [28,29]. A positive Phase I clinical study with the inhibitor DNL201, developed by Denali Therapeutics, in 2018 further highlights the opportunity with this approach.

In addition to the kinase domain, several studies have also identified the GTPase domain as a potential target for therapeutic treatments. Some of the developed compounds not only can inhibit the GTP-binding and kinase activity of LRRK2 *in vitro*, but are also active *in vivo* and can reduce neuronal degeneration [30]. Other approaches, such as targeting the pathways that LRRK2 protein substrates are involved in, could also be useful for the development of new treatments against PD. Here, we highlight some of these alternative approaches.

In 2008, Imai *et al.* showed that LRRK2-mediated hyperphosphorylation of the translation initiator factor binding protein 4E-

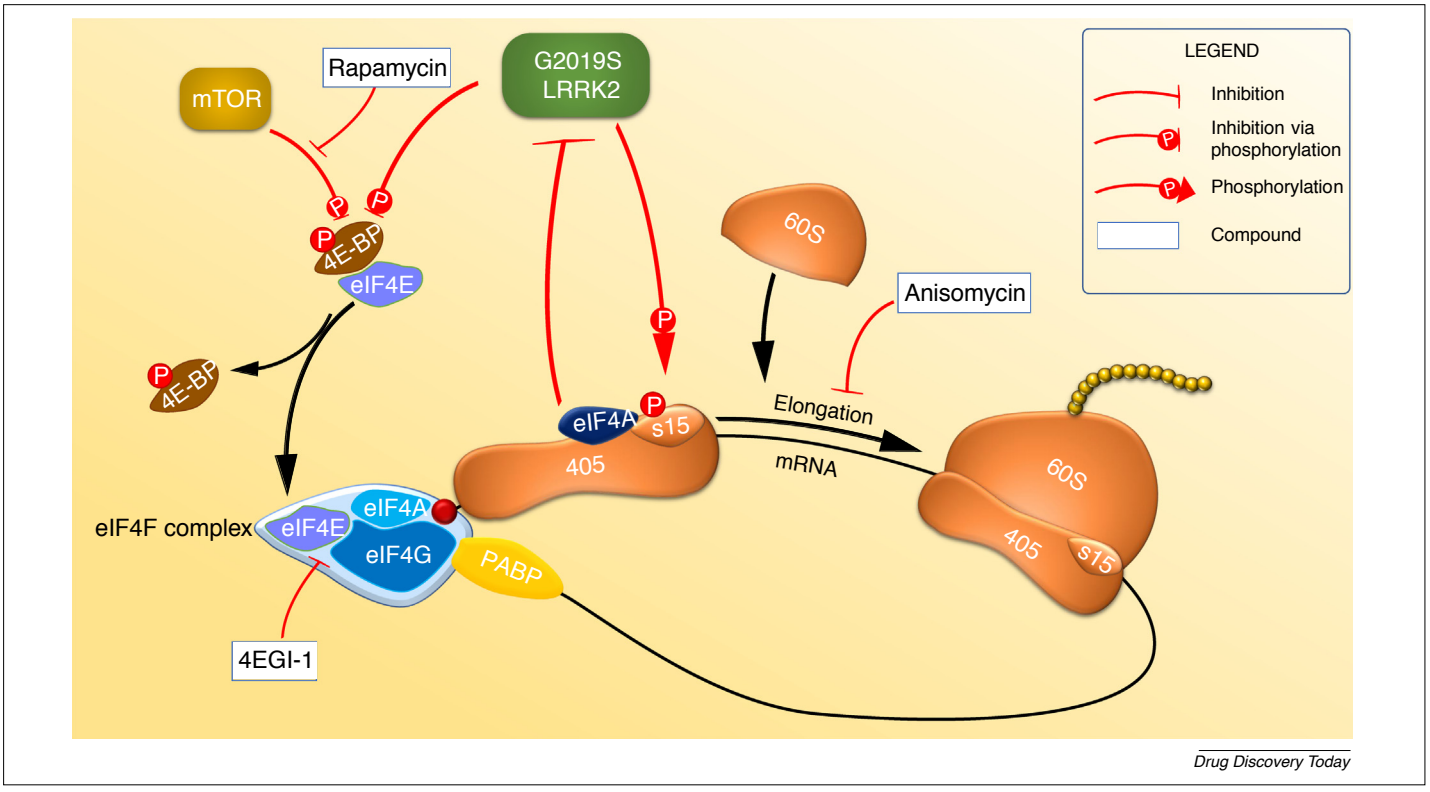

FIGURE 1

The involvement of leucine-rich repeat kinase 2 (LRRK2) in translation. G2019S-LRRK2 contributes to the hyperphosphorylation of 4E-BP. 4E-BP is also a substrate of the mTOR pathway, which can be inhibited by rapamycin. 4E-BP phosphorylation causes the release of its binding partner eukaryotic translation initiation factor 4**E** (eIF4E), which can now join the other components of eIF4F complex and activate cap-dependent translation. The interaction between eIF4E and eIF4G can be inhibited by the compound 4EGI-1. Within the ribosome, G2019S-LRRK2 phosphorylates the ribosomal protein s15, leading to an increase in both cap-dependent and cap-independent mRNA translation, which can be inhibited by anisomycin during the elongation step.

BP deregulates protein translation and affects the cellular stress response and maintenance of neuronal cells in *Drosophila* [31]. 4E-BP is a repressor of mRNA translation and limits the ability of eukaryotic initiation factor 4E (eIF4E) to bind with eukaryotic initiation factor 4 gamma 1 (eIF4G1), which are both required for translation initiation, through the formation of the 4E-BP–eIF4E complex. The binding of 4E-BP to eIF4E is regulated by phosphorylation. Hypophosphorylation of 4E-BP increases its affinity with eIF4E, whereas hyperphosphorylation of 4E-BP leads to the dissociation of eIF4E. In 2010, the same group showed that increased phosphorylation of 4E-BP1 caused by pathogenic LRRK2 inhibited the activity of miRNA in translational repression, leading to the activation of a small number of mRNAs and subsequently to the upregulation of translation [32]. However, the link between phospho-4E-BP and translation dysregulation is not clear, because there is no direct evidence showing the activation of those mRNAs are directly linked to phospho-4E-BP [33].

The regulation of 4E-BP phosphorylation in cells is not well understood. Apart from being a putative substrate of LRRK2, 4E-BP has also been shown to be a substrate of the serine/threonine kinase TOR. Tain *et al.* showed that the effect of hyperphosphorylated 4E-BP on cap-dependent protein translation can be modulated by inhibiting the TOR pathway with rapamycin [34]. However, it is not clear whether rapamycin could also rescue the pathological increase in translation in LRRK2-PD animal models. There are also contrasting evidence about whether 4E-BP is the 'authentic' LRRK2 substrate in mammalian cells [35,36]. Nonetheless, 4E-BP represents a potential target for the development of new treatments to regulate translation dysregulation and potentially PD.

Apart from targeting 4E-BP phosphorylation, another potential method to regulate translation dysregulation caused by 4E-BP hyperphosphorylation (by TOR or LRRK2) is to prevent the interactions between eIF4E and eIF4G1 so that translation cannot be initiated. This method was explored by Martin *et al.* in 2014, in which they showed that cap-dependent protein synthesis can be blocked in a G2019S-LRRK2 fruit fly PD model by the administration of the protein synthesis inhibitor 4EGI-1 [37]. The effects of 4EGI-1 on the inhibition of protein synthesis are well established [38,39]. 4EGI-1 disrupts the binding of eIF4E and eIF4G1 by binding to eIF4E. It also enhances the interaction between eIF4E and 4E-BP. The interactions between eIF4E, 4EGI-1, and 4E-BP were recently characterised by an elegant NMR spectroscopy study by the group of Wagner [40,41]. Such information will be useful for further development of 4EGI-1 into a drug candidate. Apart from its potential application in PD, the effects of 4EGI-1 have also been tested in animal models for the inhibition of protein synthesis in autism [42], X-fragile syndrome [43], and some types of cancer [44].

Apart from 4E-BP, ribosomal protein s15 has also been shown to be a substrate of LRRK2. Martin *et al.* found that LRRK2 catalyses the phosphorylation of s15 both *in vivo* and *in vitro* [33]. In their study, the authors showed that the pathogenic G2019S LRRK2 mutation increased s15 phosphorylation, leading to an increase in mRNA translation and subsequently neurodegeneration. To avoid the neurotoxic effect of G2019S LRRK2, the authors coexpressed a phosphor-deficient s15, which kept mRNA translation at physiological levels. The same effect was achieved by using anisomycin, which is a protein synthesis inhibitor. However, anisomycin does not prevent s15 phosphorylation because it works as an inhibitor of peptidyl-transferase and, therefore, does not target the cause of the dysregulation. In s15, the amino acid that is being phosphorylated by LRRK2 is a threonine in position 136, which forms part of the unstructured tail at the C terminus of the protein. Therefore, to elucidate the underlying connection between s15 phosphorylation and translational dysregulation, new studies should focus on investigating the effect of s15 phosphorylation on the structure and dynamics of the ribosome. It is not known whether LRRK2 catalyses the phosphorylation of s15 as a free protein or when s15 is part of the ribosomal complex. However, s15 is known to be important in ribosomal assembly through its interactions with assembly factors [45]. New investigations should also be conducted to study the effect of s15 phosphorylation on the mechanism and speed of ribosomal assembly.

LRRK2 has also been reported to interact with eukaryotic elongation factor 1 A (eEF1 A) *in vitro* [46]. The formation of the eEF1A–LRRK2 complex prevents the homodimerisation and autophosphorylation of LRRK2. As a result, reduced LRRK2 kinase activity is observed. Interestingly, the interactions between eEF1A and G2019S-LRRK2 were found to be similar to wild-type LRRK2. Given that the binding of eEF1A to LRRK2 reduces the kinase activity of LRRK2, we suggest that eEF1A could also lower the increased mutant LRRK2 kinase activity that is caused by the pathogenic G2019 mutation. Therefore, detailed studies should be conducted to investigate the effect of eEF1A binding to mutant LRRK2. Structural information between eEF1A and LRRK2 would also be useful so that new compounds can be developed to mimic the inhibiting binding activity of eEF1A towards LRRK2. However, the effect of LRRK2 binding on eEF1A activity in translation is not well understood. For instance, although it was shown that the binding of LRRK2 with eEF1A can modulate the function of eEF1A in promoting microtubule assembly, the physiological function of such modulation is not known.

Recently, Pellegrini *et al.* showed that the loss of LRRK2 can affect the expression of proteins that are related to mRNA translation, including translation factors [47]. In this study, it was shown that the knockout of *LRRK2* can lead to an increase in abundance of the elongation factor eEF1G. By contrast, the initiation factors eIF4G (subunit 3), eIF5, and eIF3 (subunit l) were found to decrease in their abundance. It is not known whether the changes in the amount of the translation initiation factors are directly linked to LRRK2 or whether these are downstream effects. Given the link between LRRK2 mutation and translation dysregulation, the modulation of the amount of eukaryotic initiation factors by LRRK2 is an area of significant interest for future studies.

DJ-1

DJ-1 is a multifunctional protein that is highly conserved in different domains of life. Although its principal function is poorly understood, DJ-1 is known to protect neurons from oxidative stress [48]. Mutations in *DJ-1* have been linked to PD for more than 15 years and were first reported by Bonifati *et al.* in 2003, who found that some forms of Parkinsonism are associated with deletion and missense mutations in the *DJ-1* gene [49]. DJ-1 mutations lead to a loss of its function, and subsequently cause degeneration of neuronal cells. Bonifati *et al.* hypothesised that

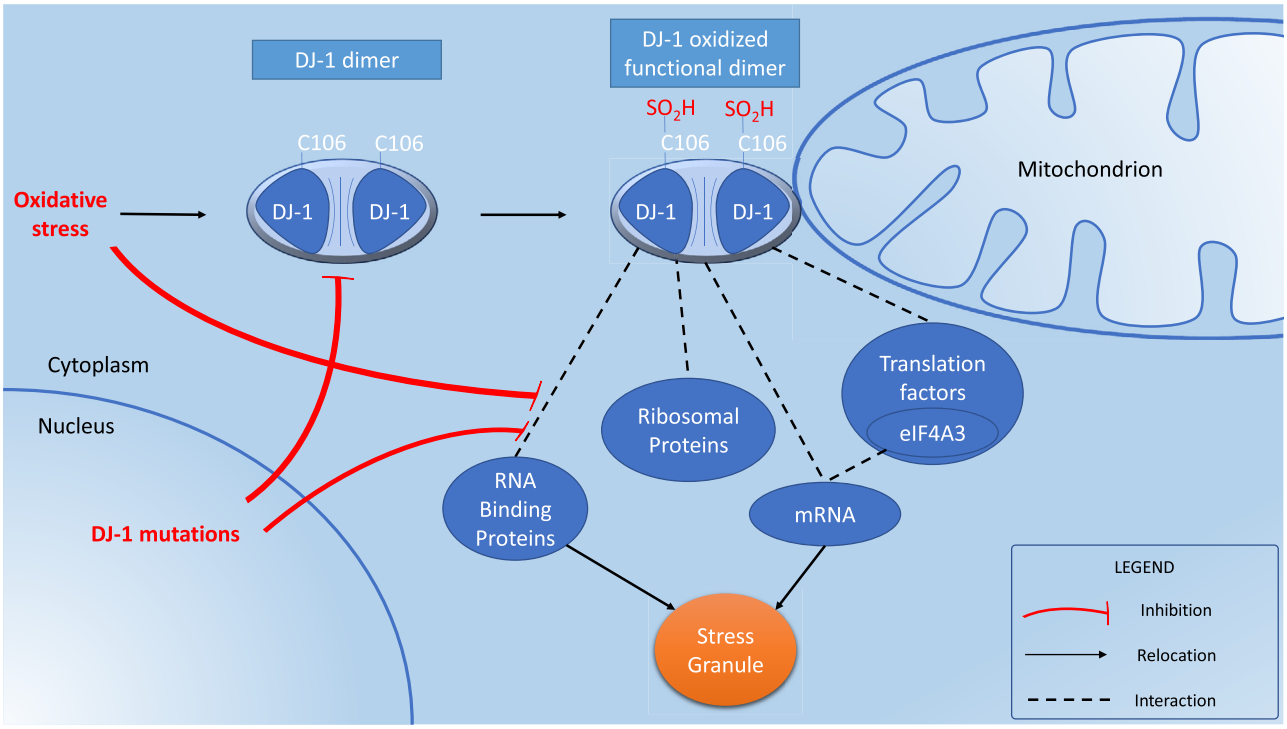

FIGURE 2

The involvement of DJ-1 in translation. Under oxidative stress, DJ-1 monomers form dimers. As a result, C106 is oxidised and dimers are relocated on the mitochondrial surface to protect neurons. Pathological *DJ-1* genetic mutations inhibit the ability to form dimers. Active DJ-1 interacts with RNA-binding proteins (RBPs) and drives these to stress granules (SGs). These interactions are lost under excessive oxidative stress conditions, and pathological *DJ-1* genetic mutations. Furthermore, DJ-1 also interacts with mRNA, ribosomal proteins, and translation factors. More specifically, DJ-1 and eukaryotic translation initiation factor 4A3 (eIF4A3) bind specific mRNAs and together drive these to SGs.

these mutations impact upon the ability of DJ-1 to interact with RNA-binding protein (RBP) complexes, as well as other factors that are involved in transcription and post-transcription, thereby preventing DJ-1 from performing its function as an oxidative stress sensor (Figure 2).

The function of DJ-1 is controlled by post-translational modification. The post-translational attachment of a sulfinic acid to cysteine-106 of DJ-1 was found to be important for driving the protein into mitochondria, where it plays out its role in neuroprotection to oxidative stress [50]. Post-translational modification of cys-106 is crucial for the function of DJ-1. For example, moderate concentrations of nitric oxide (NO) can cause *S*-nitrosylation of cysteine residues (predominately on Cys-106) on DJ-1. Given that the neuroprotective role of DJ-1 is an effect of the modulation of several signalling pathways, including the one of PTEN/PI3K/Akt [51], Choi *et al.* studied the effect of Cys-106 *S*-nitrosylation on the function of DJ-1. They showed that *S*-nitrosylated-DJ-1 transfers its NO group to the phosphatase and tensin homolog (PTEN) protein, inhibiting its activity and providing neuroprotective effects [52]. The neuroprotective effect of DJ-1 was absent when the C106 nitrosylation/oxidation site was mutated in PD models, confirming the crucial role of post-translational modifications of its Cys-106.

In 2008, van der Brug *et al.* confirmed the ability of DJ-1 to bind RNAs in an oxidation-dependent manner. The binding of DJ-1 with RNAs was found to be important for the regulation of mRNA translation of specific targets, including those that are linked to oxidative stress response (e.g., glutathione metabolism) and the cell cycle and/or apoptosis (e.g., PTEN) [53]. Interestingly, it was also shown that DJ-1 can lose its RNA-binding ability under severe oxidative stress or through its pathogenic recessive mutations. A subsequent study from the same group found that the protein products of some mRNA targets of DJ-1 were altered in the cortex of patients with sporadic PD [54]. More specifically, they found an increase in the protein levels of DJ-1 targets of the prosurvival and antioxidative pathways, whereas the level of protein targets related to mitochondrial oxidative phosphorylation decreased. However, mRNA expression levels were not changed. Although the links between translational regulation by DJ-1, post-transcription regulation of these proteins, and PD are unclear, these results nonetheless suggest that DJ-1 has a role in the response of cells to PD through its role in the oxidative stress response and mRNA transcription.

Recently, Repici *et al.* showed that DJ-1 also associates with mRNAs of translation factors, and that these are targeted to stress granules (SGs) in the cytoplasm [55]. Through mass spectrometry analysis, DJ-1 was found to interact with several proteins, including translation factors and 40S ribosomal proteins, thus suggesting a direct role in translation and its regulation. In addition, co-immunoprecipitation experiments validated the interaction between DJ-1 and the translation factor eukaryotic translation initiation factor 4A3 (eIF4A3), which is a core component of SG. The

authors proposed that DJ-1 and eIF4A3 associate with the same mRNAs, and target them from the nucleus to SG in the cytoplasm under stress conditions. However, the downstream effect of those interactions remains undefined.

The interaction of YajL, the bacterial homolog of DJ-1, with ribosomal proteins has been reported previously by Kthiri *et al.*, who first suggested a direct role of DJ-1 in translation [56]. In that study, YajL was found to interact with ribosomal proteins involved in the ribosome biogenesis, as well as with others with a direct role in translation. Under induced oxidative stress, a strain expressing a mutant form of YajL (*yajL*) showed higher dissociation of the ribosomal subunits 50S and 30S compared with the wild type. Furthermore, a decreased translation accuracy of *yajL* was found, with a consequent increase in the events of –1 and +1 frameshifts. A study in 2018 showed that wild-type DJ-1, but not its mutant M26I DJ-1, promoted neuronal health against oxidative stress by modulating the expression of miR221 [57]. When DJ-1 was mutated, there was an uncontrolled level of miR221 expression, leading to an increase in apoptotic proteins and, thus, cell death. However, the molecular mechanisms are not clear and further investigations are needed to elucidate this aspect and validate the findings in *in vivo* PD models.

Given the link between DJ-1 mutation and PD, this pathway has been investigated for the development of new treatments against PD. However, because DJ-1 mutations lead to a loss-of-function of the protein, it is difficult to design small molecules that could rescue the function of DJ-1. To date, high-resolution structures of DJ-1 only provide details about its 3D arrangement [58,59]. It would be useful to apply structural techniques to investigate the interactions between DJ-1 and its target mRNAs and proteins. Such experiments could identify amino acids that are responsible for DJ-1 binding and regulatory activity, which would be useful for the design of molecules that compensate loss-of-function DJ-1 mutation. As a proof-of-principle study, a small peptide that was designed bioinformatically based on the conserved sequence of DJ-1 was shown to enhance the protecting activity under oxidative stress conditions *in vitro* [60,61]. However, further studies are needed to investigate the applicability and stability of such peptides (or their analogues) both in cells and *in vivo*.

From a structural point of view, it is known that DJ-1 works as a dimer. Some clinical mutations of DJ-1 prevent the protein from dimerisation and lead to a loss of its neuroprotective function with rapid degradation [62–64]. As described earlier, post-translational modifications of a cysteine residue in position 106 of DJ-1 were crucial for its role in neuroprotection against oxidative stress [50]. However, increased levels of C106 oxidation can also lead to a loss of DJ-1 function. Such structural and functional information is important for the development of new drugs against PD [48,65]. For example, new compounds have been designed and tested *in vitro* and *in vivo* in PD model rats to prevent excessive cysteine oxidation [66,67]. These compounds were able to bind the C106 region of DJ-1 and protect the cysteine from excessive levels of modification, thus preventing the death of stress-induced cells and improving movements of PD-model rats. In the same way, several DJ-1 binding compounds with neuroprotective effects have been designed and tested *in vitro* and in *in vivo* PD animal models [67–70]. In addition to synthetic compounds, some natural molecules screened from traditional Chinese medicine (TCM) were also found to protect neurons from oxidative stress in synergy with DJ-1 [71]. However, these studies did not correlate the neuroprotective effects of the compounds with mRNA translation. By contrast, increased levels of DJ-1 itself were shown to modulate mRNA and protein levels in PD animal models [72]. In that study, recombinant DJ-1 was used as a drug itself by injection into two different PD rat models. The expression of proteins involved in mitochondrial oxidative phosphorylation increased significantly, whereas the pathogenic increase in mRNA and protein levels of α-synuclein decreased. As a result, dopaminergic neurons were protected with a consequent improvement in PD phenotypes. However, in this study, the molecular mechanisms of regulation were not determined, and it is not clear whether protein levels were modulated directly at a translational level or only indirectly at the transcriptional level. Other studies reported the same modulatory effect after an increase of DJ-1 levels *in vitro* and *in vivo* achieved by upregulating *DJ-1* expression using the histone deacetylase inhibitor phenylbutyrate [73] and the natural metabolite of cinnamon, sodium benzoate [74,75]. Finally, because the number of known gene products regulated by DJ-1 is limited, we believe that new high-throughput studies should focus on the identification of new DJ-1 targets to give insights into the pathology of PD.

PINK1/Parkin pathway

Two of the known recessive forms of Parkinsonism are caused by rare mutations in the genes *PINK1* and *Parkin*. These genes encode two proteins, PINK1 and Parkin, the main role of which usually involves autophagy (reviewed in [76]). In fully functioning mitochondria, PINK1 mainly locates on the outer membrane, with a short portion of its N-terminal imported into the inner membrane. This is then cleaved and remains in the mitochondrion, while PINK1 is released and degraded. By contrast, PINK1 accumulates on the outer membrane of damaged mitochondria, recruiting Parkin, which can induce mitophagy [77]. It has also been suggested that PINK1/Parkin protect neurons against oxidative stress and dysfunctions of the mitochondria through quality control pathways that involve mRNA translation. A few studies conducted in *Drosophila* revealed that interactions between proteins in the PINK1/Parkin pathway and proteins that are involved in translation, such as 4E-BP [34], ribosomal protein S6 kinase (S6K) and eIF4E [78], might be important in PD (Figure 3).

In the first study, it was found that overexpression of the translation inhibitor 4E-BP could prevent neurodegeneration in *Drosophila* that carry *PINK1/Parkin* mutations (see earlier). In the second study, S6K was found to be a sstrong modulator of the toxic effects that are caused by *PINK1* mutations. More specifically, it was found that activation of S6K can lead to translation enhancement, which aggravates *PINK1* mutant phenotypes, whereas these effects were suppressed in S6K knockdown (which reduced protein translation). In the same way, eIF4E overexpression leads to a worsening of *PINK1* mutant phenotypes. These results indicate that cap-dependent translation is upregulated in *PINK1* mutants and any tentative downregulation would be useful to prevent the degeneration of cells.

A subsequent study in mice investigated the role of PINK1 under hypoxia and indicated PINK1 as a modulator for cap-dependent and cap-independent translation, as well as an activator of the transcriptional factor HIF-1α pathway [79]. In that study, the

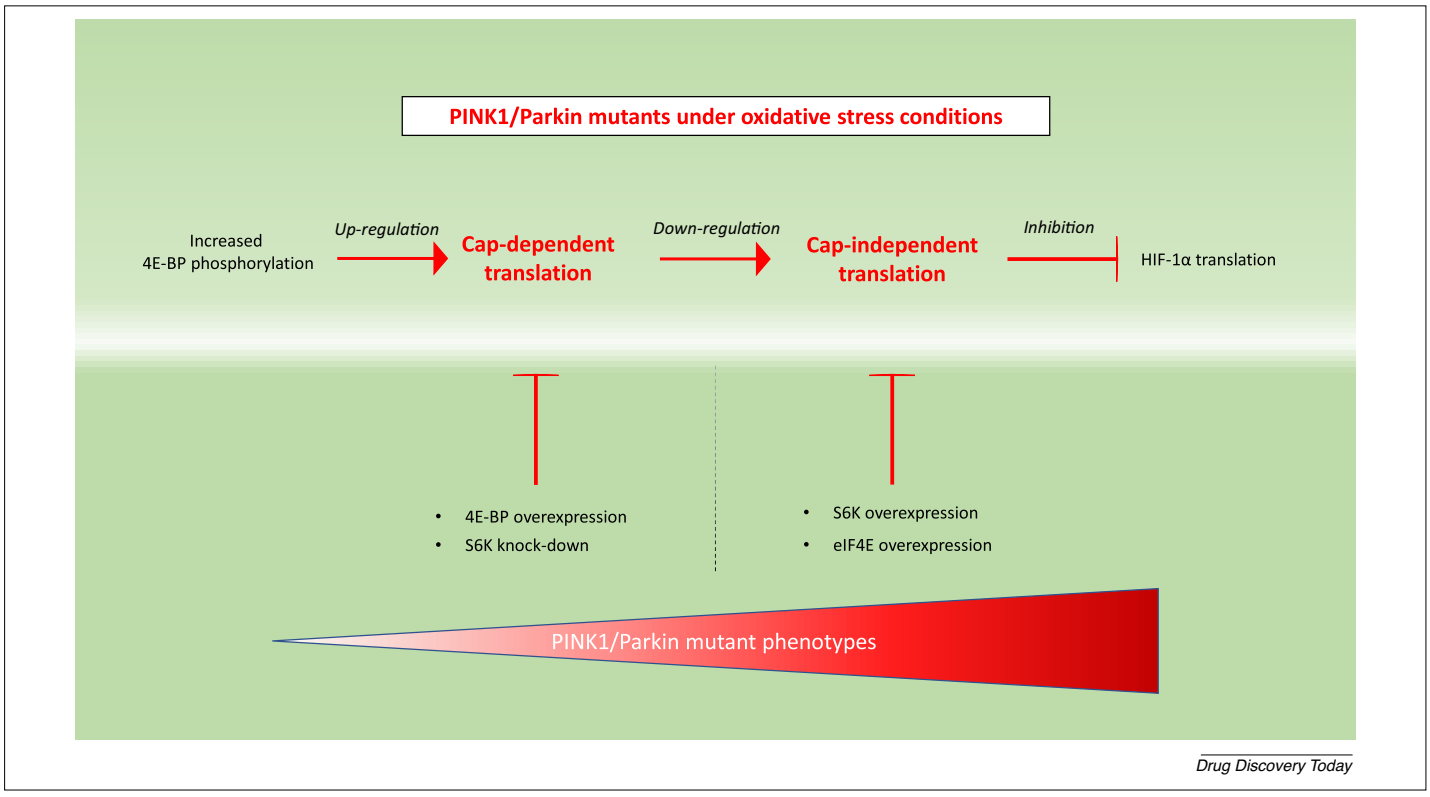

FIGURE 3

PINK1 and Parkin mutants under oxidative stress conditions. Under oxidative stress conditions, there is an increase in production of HIF-1α, the mRNA of which is translated in a cap-independent manner. In PINK1/Parkin mutants, there is hyperphosphorylation of the translation inhibitor 4E-BP, which causes the up-regulation of cap-dependent translation and a consequent down-regulation of cap-independent translation. As a result, there is a decrease in translation of HIF-1α mRNA. PINK1/Parkin mutant phenotypes can be rescued by overexpression of 4E-BP and in S6K knockdown. This is probably because of the inhibition of cap-dependent translation. By contrast, the overexpression of S6K and the translation factor eIF4E lead to a worsening of PINK1/Parkin mutant phenotypes. This is probably because of the upregulation of cap-dependent translation, which implies the downregulation of cap-independent translation.

synthesis of the transcriptional factor HIF-1α under hypoxia, which, under that condition is likely to be translated in a cap-independent manner, was decreased in *PINK1* mutants. This correlated with an increase in 4E-BP activation by phosphorylation, which implies the upregulation of cap-dependent translation (Fig. 3).

In 2015, Gehrke *et al.* revealed a new role of PINK1 and Parkin in RNA metabolism in flies and mammalian cells [80]. It was found that PINK1 (as well as a translocase of outer membrane, Tom20) are localised on the mitochondrial outer membrane and are involved in the translation of specific mRNAs for components of the mitochondrial respiratory chain. By contrast, in *PINK1* mutants, the translation of certain components was reduced specifically in neuromuscular tissue. Furthermore, PINK1, but not its mutant version G309D-PINK1, interacts with the 5′ cap of specific mRNAs through interactions with the translation initiator factor eIF4G. By interacting with mRNAs, PINK1 competes with translational repressors, including Pumilio, and, with Parkin, promotes the displacement of such repressors. Interestingly, the G309D mutation affects the PINK1 kinase domain, and it is possible that the PINK1–eIF4G interaction is kinase dependent. The *PINK1* mutant phenotype could be rescued in different ways, including the use of translational repressors with RNAi or by overexpressing Tom20. At the same time, PINK1 overexpression was found to rescue a loss of Tom20, suggesting that PINK1 and Tom20 work together as a complex.

A recent study from the same research group elucidated at molecular levels the connection between the regulation of mRNA translation and the mitochondrial quality control pathway, mechanisms that are coordinated by PINK1 [81]. Under stress conditions, there is an increase in the translation of oxidative phosphorylation-related mRNAs, which is carried out by ribosomes located on the mitochondrial outer membrane in a PINK1-mediated manner. In damaged mitochondria, mRNA translation stalls, thus causing the attraction toward the ribosome of co-translational quality control components, which generate signals to trigger autophagy of faulty mitochondria (mitophagy). In this study, the authors provided insights into the connection between mitochondrial damage and altered proteostasis. However, the way in which these mechanisms are related to the process of neurodegenerative diseases remains unclear. The exact molecular mechanisms that cause ribosome stalling on damaged mitochondria are also unclear, including whether these mechanisms are correlated to mutations in *PINK1* and *Parkin*. Overall, two different roles of mRNA translation in PINK1-related PD pathogenesis can be distinguished: (i) the reduction in a highly energy-demanding process, such as protein translation, is beneficial for the survival of the energy-compromised PINK1 PD models; and (2) the stimulation of translation of mRNA encoding respiratory chain components can restore mitochondrial function in PINK1 PD models.

So far, only a few molecules have been shown to suppress the pathogenic effects of PINK1 loss or its mutated forms. In 2006, a

diet supplemented with antioxidants, including the recombinant human antioxidant enzyme SOD1 and vitamin E, was shown to protect *PINK1*-knockdown neurons in *Drosophila* [82]. In 2013, a method of direct PINK1 activation was described by Hertz *et al.* [83]. In this study, the authors reported a strategy to enhance the activity of PINK1 and to activate the mutant G309D-PINK1 by using the ATP analogue kinetin-triphosphate (KTP) or its precursor kinetin in *in vitro* assays and in human cell cultures. However, a subsequent study from the same group did not confirm the biological activity of long-term kinetin administration in PD model mice and showed that kinetin could not prevent neurodegeneration in α-synuclein PD models [84]. In 2017, another research group followed up on this work and developed a method for the synthesis of four kinetin metabolites [85]. Kinetin riboside prodrug-nucleotides (Pro-Tides) were shown to be stable in mouse and human serum as well as in acidic environments, and to activate PINK1 more efficiently in human cells compared with kinetin. Kinetin metabolite are not inhibitors of the PINK1 kinase domain, and are considered neosubstrates. Future studies should also focus on the effects of these metabolites on the proteolytic processing of PINK1 for mitochondrial entry, targeting, and subsequent degradation. In 2018, two new classes of molecule were tested in terms of their ability to activate PINK1. Barini *et al.* showed that the anthelmintic drug niclosamide and analogues of this molecule can indirectly activate the PINK1/Parkin pathway in neuronal cells [86], whereas Biosa *et al.* designed SOD-mimetic compounds that can be used against the toxicity caused by a loss of PINK1 and Parkin [87]. Interestingly, mitochondrial dysfunction and death of PINK1-deficient cells could be rescued by curcumin by modulating the intracellular level of calcium and the mitochondrial membrane potential [88].

Other targets

EIF4G1 and VPS35

In 2011, mutations in the gene *EIF4G1* were first found in a French family affected by autosomal-dominant late-onset PD [89]. This gene encodes the translation initiation factor eIF4G, which is one of the key components in the formation of the cap complex. These mutations suggested potential eIF4G dysfunctions, which could lead to faulty translation of existing mRNAs in neurons under stress conditions. However, mutations in *EIF4G1* were found to be rare in other case studies and, if present, would not be involved in PD aetiology [90,91]. Despite this controversy, eIF4G was found to genetically and functionally interact with the PD-related genes *SNCA* and *VPS35* in yeast and *Caenorhabditis elegans* neurons [92]. In that study, the overexpression of eIF4G, but not of the PD-associated variant R1205H, protected against α-synuclein toxicity in yeast, supporting the role of eIF4G mutants in PD. Furthermore, the yeast homolog of VPS35 was found to protect against toxic effects because of the upregulation of the homologue of eIF4G. However, these latest findings might not be relevant because there is no evidence of eIF4G upregulation in PD. Instead, it would be interesting to confirm in PD-models whether eIF4G mutant-altered affinity with other components of the eIF4F complex, as well as other initiation factors, can affect cap-dependent and cap-independent translation. Furthermore, as described earlier, eIF4G interacts with PINK1 and this interaction is attenuated when PINK1 is mutated. It would be interesting to determine whether this loss of affinity can be reciprocal by testing whether mutant variants of eIF4G lose their interaction with PINK1 and other cellular components compromising mRNA translation of mitochondrial components.

VPS35 is a subunit of a heteropentameric complex called retromer, which is responsible for the recycling and transport of transmembrane receptors from the endosome to the *trans*-Golgi network and the cytoplasmic membrane [93]. In 2011, mutations in the *VPS35* gene were first found in European families with PD. Since then, the D620 N mutation has been found in patients with PD worldwide [94,95]. Studies of the effects of mutant variants of VPS35 in PD (reviewed in [96,97]) have shown that only the D620 N mutant is pathogenic. Although D620N-VPS35 causes neurodegeneration in animal PD models, this aspartate to asparagine substitution does not alter the interaction of VPS35 with other retromeric components [98]. A few cellular mechanisms are disrupted by D620N-VPS35, but none of these is found to be directly involved in neurodegeneration [96]. In addition to its interaction with *EIF4G1* and *SNCA* [92], VPS35 was found to genetically and functionally interact with *LRRK2* [99] and *Parkin* [100], which are also involved in protein translation. Overexpression of the wild-type VPS35, but not D620N-VPS35, could overcome the dysfunction in protein sorting caused by defects in the LRRK2-RAB7L1 pathway [99]. Recently, Williams *et al.* showed that Parkin has a role in protein trafficking via the ubiquitination of VPS35. Overall *VPS35* mutations might cause dysfunction in protein trafficking, recycling, and degradation, and these contribute to the hypothesis that altered protein homeostasis is crucial in PD.

Concluding remarks

Translation is a complicated mechanism that can be divided in four stages: initiation, elongation, termination, and recycling. During initiation, the ribosome is assisted by initiation factors to form a complex with mRNA and the first tRNA. During elongation, elongation factors assist the ribosome to carry out its functions, including the reaction of peptide bond synthesis. Finally, release factors drive the termination of the process mediating the release of the peptide chain and mRNA from the ribosomal subunits, which can dissociate and begin a new cycle. Several regulation events allow this mechanism to be carried out correctly and in proper locations, ensuring protein homeostasis and cell vitality. In PD, a combination of environmental factors and many different genetic mutations result in various pathological mechanisms that lead to cellular dysfunctions. Mutations in most of the PD-related genes cause dysfunctions in different ways, but it is becoming clear that many of these converge on altering the process of mRNA translation and its regulation, which appears to have a key role in PD pathogenesis. The centrality of mRNA translation in PD brings up the need to find alternative routes for PD therapies. Several aspects of the relationship between PD and translation need to be investigated in depth to further understand the molecular mechanisms behind PD pathogenesis. So far, none of the studies that tested chemical compounds in PD animal models aimed to understand their effect on translation. Some of those compounds could be a good starting point for new tests, both *in vitro* and *in vivo*. However, much effort is also needed in developing new chemical compounds that are specifically

designed for restoring mRNA translation functionality in different types of PD model, with the ultimate goal of providing new therapeutic tools for patients with PD.

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