

***Structure and function of Fbxo7/PARK15 in Parkinson's disease.***

Suzanne J. Randle<sup>1</sup> and Heike Laman<sup>1</sup>

Author affiliations:

1 Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK.

Author for correspondence: Heike Laman; e-mail [hl316@cam.ac.uk](mailto:hl316@cam.ac.uk)

Key words: Fbxo7/PARK15, F-box protein, SCF-ligase, ubiquitin, E3 ligase, Parkinson's disease, mitophagy

**Abstract**

Fbxo7/PARK15 has well-defined roles, acting as part of a Skp1-Cul1-F box protein (SCF)-type E3 ubiquitin ligase and also has SCF-independent activities. Mutations within *FBXO7* have been found to cause an early-onset Parkinson's disease, and these are found within or near to its functional domains, including its F-box domain (FBD), its proline rich region (PRR), and its ubiquitin-like domain (Ubl). We highlight recent advances in our understanding of Fbxo7 function in Parkinson's disease, with respect to these mutations and where they occur in the Fbxo7 protein. We hypothesize that many of Fbxo7 functions contribute to its role in PD pathogenesis.

Parkinson's disease (PD) is the second most common neurodegenerative disease; it has been estimated that in 2005, the number of individuals over age 50 afflicted with PD was between 4.1 and 4.6 million in the 15 most populous countries in the world [1]. The majority of PD cases are sporadic and idiopathic with a median age of onset of 68. Men stand a greater risk of developing PD [2, 3], and some genetic factors and environmental influences, like head trauma, exposure to pesticides, and certain foods, are also associated with a higher risk for PD [4]. Currently, there are no good diagnostic biomarkers for the early stages of disease, and there is also no cure.

PD is characterized by the loss of dopamine (DA)-producing neurons in the substantia nigra pars compacta, although pathology is not restricted to this site. Diagnosis depends on the presence of specific clinical symptoms and signs including a resting tremor, rigidity, bradykinesia, and postural instability, and is often accompanied by an array of non-motor features, only some of which take their origin from the dopaminergic nigrostriatal pathway. Post-mortem, the disease is characterized histopathologically by the presence of intra-cytoplasmic protein aggregates known as Lewy bodies (LBs), which contain  $\alpha$ -synuclein among other proteins.

However, PD is not exclusively a disease of old age. Approximately 5-10% of sufferers are under the age of 40, and among these patients there are a discrete number of genes that have been linked to the development of disease [5], and which are the subject of other articles in this issue. One example of a rare mutation that is associated with a juvenile Parkinsonian-pyramidal disease (PPD) is *PARK15*, more commonly known as *FBXO7* (F-box protein only 7). The clinico-pathological symptoms of PPD (otherwise known as Pallido-pyramidal disease) first described by Charles Davison in 1954, suggested involvement of neuronal pathways that descend through the pyramidal tract, including Babinski sign and hypertonic spasticity [6]. These are distinct from the extra-pyramidal symptoms characteristic of PD, such as those listed above, which were also evident in these patients. However, more recently Horstink, *et al.* have brought into question the classification of PPD as a distinct clinical syndrome by suggesting that most pyramidal signs can be explained by other affected pathways; an idea supported by a lack of pallido-pyramidal degeneration in PPD patients [7]. A full pathological description of *FBXO7* PPD is as yet unavailable, but these patients are Parkinsonian and respond to L-Dopa, and so must have pathology that is relevant to PD [8]. In addition, Fbxo7 protein is found in LBs of patients with sporadic disease, hinting at its involvement in idiopathic cases [9]. So although Fbxo7-associated disease is rare, research into this monogenic disease may reveal pathological changes that also underlie the development of sporadic PD. This review will describe the mutations in *FBXO7* that are associated with disease to date, the functional domains in which they occur, and the insight they give into the potential mechanisms of neurodegeneration.

### **Pathogenic mutations in *FBXO7* map to particular functional domains**

Disease-linked mutations in *FBXO7* have now been found in several families from countries across Europe and the Middle East [10-14]. These mutations cause a juvenile-onset PD frequently with pyramidal tract symptoms, such as Babinski sign and spasticity [12]. Other symptoms in *PARK15* patients can also include tics and chorea, expanding the phenotypic spectrum associated with its mutation [13]. Mutations are inherited recessively, suggesting they produce a hypomorphic protein or cause a loss of function. Whereas most research on Fbxo7, prior to the first PD related report in 2008, investigated its role as a cell cycle and proteasome regulator, these findings raise questions about its cell-type specific

role in neurons. The use of high density whole-genome SNP arrays on an Iranian family, several members of which have Parkinsonian-pyramidal syndrome (PPS), enabled the discovery of the first homozygous missense mutation within *FBXO7* (p.R378G) [12]. Other mutations reported shortly thereafter (Table 1), include a homozygous truncating p.R498X mutation in Italian, Turkish and Pakistani families [10, 11], and a compound heterozygous splice-site variant (IVS7 + 1G/T) and point mutation (p.T22M) in a Dutch family [10]. In the above instances, both copies of *FBXO7* are mutated, but single allele changes have also been found. For example, a heterozygous p.R481C substitution has been found in an Italian family with homozygous p.G877R mutation in *PARK9* [15]. In addition, single heterozygous amino acid substitutions (p.I87T and p.D328R) are present in two cases of early-onset PD in Taiwan [16].

Table 1. List of protein coding *FBXO7* SNPs associated with PD or other clinical parameters.

Mutation	Domain	Notes	References
T22M	Ubl	- Compound heterozygous mutation with splice site IVS7 + 1G/T mutation found in Dutch family - Compromises Parkin binding and cellular location, as well as reducing expression levels when present with IVS7 + 1G/T.	[10, 45, 46]
Y52C	Ubl	- Potentially protective against PD in Chinese and Taiwanese populations.	[17]
I87T	Near Ubl	- Found in early onset Taiwanese PD patient. - No functional data as yet.	[16]
M115I	Near CDK6	- Associated with altered RBC parameters. - No association with PD.	[17-19]
D328R	Between FP and F-box	- Found in early onset Taiwanese PD patient. - No functional data as yet.	[16]
R378G	Near F-box domain	- Homozygous mutation found in Iranian family. - Compromised Skp1 binding and altered location. - No effect on NF-κB signalling.	[12, 27, 37]
R481C	R(Ar)DP motif within PRR	- Heterozygous compound mutation found with homozygous G877R <i>PARK9</i> mutation in Italian family. - No functional data as yet.	[15]
R498X	PRR	- Homozygous truncating mutation found in multiple families. - Reduced protein expression seen in patients fibroblasts. - No effect on NF-κB signalling. - Reduced capacity to recruit Parkin to mitochondria.	[10, 11, 13, 14, 27, 45, 46]

Other SNPs in *FBXO7* have also recently been identified in relation to idiopathic PD; an allele encoding for p.Y52C is associated with *decreased* risk of disease in Chinese and Taiwanese patients [17], whereas a non-coding IVS-329C>T variant is moderately associated with *increased* risk [16]. Other GWAS studies have identified SNPs in *FBXO7* as being relevant for

measurable erythroid parameters, including one that causes a coding substitution (p.M115I) [18]. However, no significant association between this SNP and PD has been found [17, 19].

Mapping these pathogenic mutations and associated SNPs to different *FBXO7* exons indicates that many of its functional protein domains may be affected to cause pathology; however, most of these alterations cluster in or around the N-terminal ubiquitin-like (Ubl) domain, or the C terminal F-box domain (FBD) and proline rich region (PRR) (Figure 1A). These changes in relation to function (Figure 2) will be discussed below.

### **The F-box domain of Fbxo7**

The F-box domain within Fbxo7 enables its interaction with Skp1 and was originally identified using bioinformatics and functional approaches [20-22]. The presence of this three  $\alpha$ -helical motif defines Fbxo7 as an F-box protein (FBP), and allows Fbxo7, and presumably any FBP, to become part of a Skp1-Cul1-FBP (SCF) type E3 ubiquitin ligase. An exemplar crystal structure of the F-box domain of Skp2 in complex with the other SCF components is shown in Figure 1D [23]. Within Fbxo7 isoform 1, its F-box domain is approximately two thirds along the length of the primary sequence, spanning amino acids 335-372. Fbxo7 is the fifth most abundant SCF ligase in cultured cells, as assayed by quantitative mass spectrometry analysis of CUL1 binding partners [24]. This abundance notwithstanding, to date there are just four published substrates of SCF<sup>Fbxo7</sup>. These include Hepatoma UpRegulated Protein (HURP) [25] and the NF- $\kappa$ B signaling pathway proteins, cIAP1, TRAF2 and NRAGE [26-28]. The first target of Fbxo7 mediated ubiquitination to be identified was HURP, a mitotic spindle protein that can regulate chromosome congression and is a proto-oncogene in hepatocellular carcinoma [29, 30]. Ubiquitination of HURP by SCF<sup>Fbxo7</sup> leads to its proteasomal degradation. However, unusually for an F-box protein, ubiquitination of the remaining substrates by SCF<sup>Fbxo7</sup> does not lead to their degradation, suggesting that alternative ubiquitin chains are formed on these substrates rather than the canonical K48-linked poly-ubiquitination that marks proteins for proteasomal degradation. In fact, it has been shown that poly-ubiquitination of NRAGE (Neurotrophin Receptor-Interacting MAGE Homolog) by SCF<sup>Fbxo7</sup> is via K63-linked ubiquitin, indicating that SCF<sup>Fbxo7</sup> is able to catalyze different types of ubiquitin chain linkages [28]. Further research is required to determine what governs these differences that so profoundly affect the fate for SCF<sup>Fbxo7</sup> substrates. It is possible that it may be due to differences in the manner in which substrates engage with the SCF<sup>Fbxo7</sup> ligase or its ability to recruit a variety of E2 enzymes that dictates a particular chain linkage. It will also be interesting to determine whether cIAP (cellular Inhibitor of Appoptosis) and TRAF2 (TNF $\alpha$  Receptor Associated Factor 2) are modified by K63-linked ubiquitin chains impacting their function. These ubiquitination events generally lead to changes in a substrate's function, localization and/or binding to other effectors [31]. In addition, poly-ubiquitination of proteins by K63-linked ubiquitin chains can also lead to their degradation in a proteasome-independent manner. For example, p62/SQSTM1 and ESCRT0 complexes preferentially bind K63-linked poly-ubiquitinated proteins to target them to lysosomes, leading to their degradation via autophagy [32, 33]. In the case of NRAGE, Fbxo7 facilitates its ubiquitination to promote its inclusion into active NRAGE-TAK1-TAB1 complexes, leading to elevated NF- $\kappa$ B signaling [28]. The inflammatory NF- $\kappa$ B pathway, implicated in PD and other neurodegenerative diseases such as Alzheimer's disease, is highly dependent on ubiquitination for its coordination [34]. Many proteins in this pathway are ubiquitinated, with various consequences, including degradation of inhibitors and cleavage of precursor proteins to their active forms via the proteasome, and activation of

protein complexes such as inhibitor kappa B kinase (IKK). Unlike NUAGE, the functional consequence of cIAP1 and TRAF2 ubiquitination by Fbxo7 is inhibition of NF- $\kappa$ B signaling [27]. Upon tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) stimulation, cIAP1 and TRAF2, which are both E3 ligases, are recruited into TNF-receptor signaling complexes (TNF-RSC), leading to K63-linked ubiquitination of receptor interacting protein 1 (RIP1) activating the IKK signalosome [35, 36]. However, SCF<sup>Fbxo7</sup> ubiquitination of cIAP1 and TRAF2 decreases their ability to ubiquitinate RIP, thereby inhibiting NF- $\kappa$ B signaling [27]. Although aberrant NF- $\kappa$ B signaling suggests an intriguing potential explanation for Fbxo7 pathogenesis, initial investigation of mutant R378G and R498X alleles of Fbxo7 showed no effect on NF- $\kappa$ B signaling [27]. Why Fbxo7 both positively and negatively regulates NF- $\kappa$ B signaling remains unclear. These differential effects may stem from cell-type specific effects, but it highlights the role of Fbxo7 as a scaffold that integrates upstream signaling events to effect disparate outcomes.

One alternate hypothesis to explain Fbxo7 pathology is that mutations in this F-box protein, which specifies protein ubiquitination, disables its enzymatic activity leading to toxic accumulation of a particular but as yet unknown protein, causing subsequent pathology. In fact, one of the homozygous mutations, p.R378G, impairs the ability of Fbxo7 to function as an FBP, by reducing its ability to bind to Skp1 [37]. This mutation results in a change in protein sequence from a positively charged arginine to a smaller, amphoteric glycine residue. As this residue falls six amino acids outside of the boundaries of the F-box domain, this change may cause a conformational change that impinges on Skp1 binding or may result in fewer potential hydrogen bonds needed to stabilize Skp1 binding. Regardless, the outcome of this reduced interaction would be to decrease ubiquitination of all Fbxo7 substrates, one or more of which is thought to be critical for PD. An additional consideration is that Nelson and Laman also found that the p.R378G mutation induces a subtle change in subcellular location of Fbxo7, causing it to be more cytoplasmic [37]. This is likely due to the requirement for Skp1 binding to Fbxo7 to promote its accumulation in the nucleus during S-phase of the cell cycle. A nuclear export sequence overlaps the start of the F-box domain, setting up a competitive relationship between Skp1 binding and binding to the nuclear export protein CRM1. Since Fbxo7 p.R378G has a reduced capacity to bind Skp1, this consequently increased binding to CRM1 causing its export from the nucleus and accumulation in the cytoplasm. Altering the concentration of Fbxo7 and by extension active SCF<sup>Fbxo7</sup> ligase in different subcellular compartments may reduce its ubiquitination of nuclear and cytoplasmic substrates and also favor its non-canonical interactions, and either or both of these outcomes may contribute to pathogenesis.

In addition to the p.R378G mutation located downstream of the F-box domain, a heterozygous p.D328R mutation was discovered in an early-onset PD patient from Taiwan [16]. This amino acid is located seven amino acids in front of the F-box domain, and near the dimerizing Fbxo7-PI31 (FP) domain (see below). This substitution changes a negatively charged aspartic acid residue to a positively charged arginine and Lin, *et al.* hypothesize that this alteration may change the biochemical properties of the protein by affecting proton translocation or protein stability [16]. Although it is not known whether this SNP is pathogenic or disease-modifying, it would be interesting to test whether it causes perturbations to Skp1, CRM1, or PI31 interactions similar to the p.R378G mutation.

### **The proline rich region (PRR) of Fbxo7**

Fbxo7 is part of the Fbxo 'other' sub-family of FBPs, differing from those containing leucine rich repeats (Fbx<sub>l</sub> family) and WD40 domains (Fbx<sub>w</sub> family). Members of the Fbxo

family contain a variety of protein interacting domains to recruit substrates, such as F-box-associated domains (FBA), RNase inhibitor-like motifs (RNI-like), and, in the case of Fbxo7, an N-terminal Ubl domain from amino acids 1-78 and a C-terminal PRR from amino acids 423-522 (Figure 1A). Initial studies investigating HURP ubiquitination and degradation found that Fbxo7 binds HURP using its PRR, suggesting this region mediates substrate interaction [25]. Mapping an interaction domain for cIAP1 has not been directly undertaken, but Hsu, *et al.* and Chang, *et al.* used Fbxo7 isoform 2 to test for the ubiquitination of HURP and cIAP1, respectively. Isoform 2 of Fbxo7 lacks the Ubl domain of isoform 1, and has an alternative 12 amino acid sequence at the start, demonstrating the Ubl is not required for binding to these two substrates.

The PRR domain is so named because of its high percentage of proline residues; 27% of the PRR is proline, compared to 6% in the rest of Fbxo7 and 6% throughout the entire human proteome [38]. Many of these proline residues are found in a PxxP formation which is a consensus sequence capable of binding Src Homology 3 (SH3) containing proteins, and may therefore potentially aid in protein-protein interactions between Fbxo7 and its substrates. The PRR is predicted to be largely unstructured, however it does contain a highly conserved R(Ar)DP motif, where Ar equals any aromatic residue [39]. A function for this motif has not yet been reported. Intriguingly, in patients with homozygous PARK9 mutations, who develop an atypical juvenile parkinsonism called Kufor-Rakeb syndrome, a heterozygous mutation in *FBXO7* targeting this conserved cluster of amino acids is also found [15]. This point mutation causes a p.R481C substitution, however the significance of this change has not yet been shown as it is also present in healthy family members. Although this SNP may be coincidental, it is possible that Fbxo7 activity may modify the disease phenotype along with other, as yet unidentified, mutations [15].

As well as mediating interactions with ubiquitination substrates, the PRR also forms part of a bi-partite binding domain for CDK6 interaction. The other region directly interacting with CDK6 is an unstructured region (amino acids 129-168) upstream of the FP domain and close to the p.M115I SNP associated with erythroid parameters. Binding of Fbxo7 to CDK6 does not change its expression levels, but rather enhances CDK6 activity, suggesting Fbxo7 acts as a molecular scaffold for cyclin/CDK complex assembly [20]. In addition to its ability to bind CDK6, Fbxo7 also interacts with a CDK inhibitor p27 [20]. p27 inhibits CDK1/2 complexes, yet also acts as an assembly factor for CDK4/6 complexes at physiological levels [40]. p27 can independently bind Fbxo7, resulting in its stabilization and elevated protein expression [20]. Fbxo7 interaction with cell cycle proteins precipitates either pro- or anti-proliferative effects, depending on the cell type (Figure 2) [20, 41, 42]. Whether this non-canonical ability of Fbxo7 to influence cell cycle proteins has any role in post-mitotic neurons remains to be determined; however Fbxo7 may impact on adult neurogenesis, which is highly dependent on CDK6 and p27 [43, 44].

One repeatedly isolated pathogenic mutation in *FBXO7* is the homozygous nonsense p.R498X mutation, which introduces a premature stop codon removing the final 24 amino acids of the protein. Studies using fibroblasts isolated from *FBXO7/PARK15* patients suggest these mutations primarily affect the levels of protein expression [45]. Patient fibroblasts with homozygous p.R498X mutations showed a lack of Fbxo7 protein expression despite normal mRNA levels, and heterozygous p.R498X fibroblasts showed reduced expression compared to control cells. It will be necessary to see if a similar protein expression pattern is evident in neurons bearing these mutant alleles. This will become feasible through the use of technologies such as CRISPR to introduce pathogenic mutations at the endogenous

*FBXO7* locus or iPS cells generated from *FBXO7/PARK15* patients differentiated into neurons. Nevertheless, these findings suggest that the p.R498X mutation translates to a loss of Fbxo7 expression and function for the patient.

In contrast to these findings in patient fibroblasts, experiments using exogenously expressed proteins in neuronal SHSY-5Y cells and a number of other cell types showed that Fbxo7-R498X is more robustly expressed than its WT counterpart, suggesting that this variant is more stable (our unpublished observations and [46]). This suggests that the C-terminus may promote Fbxo7 turnover, perhaps by providing a degron for its auto-ubiquitination, or ubiquitination by other SCF complexes. Fbxo7 has been found to interact with Parkin and PINK1 via its Ubl domain (see below), and through these interactions, facilitate Parkin recruitment and promote mitophagy. In experiments to test the mechanistic defect of individual PD-associated alleles in mitophagy, including p.R498X, it was shown that Fbxo7-R498X is unable to substitute for WT Fbxo7 in Parkin recruitment to mitochondria, despite its binding more efficiently to Parkin than WT [46]. In the same study, it was reported that Fbxo7-R498X cannot rescue Parkin loss in flies whereas WT Fbxo7 can [46]. These data suggest the C-terminus of Fbxo7 either stabilizes the interaction with Parkin at the mitochondria, or has other essential functions such as substrate recruitment and/or ubiquitination to enable mitophagy. One might predict based on these finding that patients with Fbxo7 p.R498X would be less able to utilize the PINK1-Parkin mitophagy pathway resulting in defective mitochondrial quality control. Experiments using physiologically relevant cell types and levels of expression are needed to reconcile the different findings seen in different cell culture systems.

### **The FP domain of Fbxo7**

The FP (Fbxo7/PI31) domain is a globular domain located centrally within Fbxo7 and enables its interaction with PI31, a protein that interacts directly with the proteasome. PI31 was cloned by two independent methodologies as an interaction partner for Fbxo7: via affinity purification from a column of GST fused to Fbxo7 amino acids 129-398, and in a yeast two-hybrid screen using the same sequences from Fbxo7 as bait [39]. A sequence alignment of PI31 and Fbxo7 revealed their relatedness at a sequence organization and structural level. They have an equivalent FP domain, from 180-324 in Fbxo7 and 1-151 in PI31, and a PRR with extended secondary structure at their C-termini. As mentioned above, the PRR of Fbxo7 is thought to play a role in substrate recruitment. Within PI31, a conserved HbYX motif (Hb=hydrophobic; X=any amino acid), which is usually present in proteasome activators, is located at the extreme end of its PRR domain and mediates its binding to the  $\alpha$ -subunits of the 20S barrel [47-49]. This motif is not present in the PRR of Fbxo7. PI31 also contains the highly conserved R(Ar)DP motif found in Fbxo7, also within its PRR, highlighting the sequence similarities between the two proteins.

### **The function of PI31 and its interaction with Fbxo7**

The function of PI31 has been tested in studies in mammalian cells, yeast, and flies; however, there appear to be disparities in its function when tested in different assays. For example, PI31 demonstrates inhibitory activity towards the 20S proteasome when tested *in vitro*, stimulatory effects on 26S proteasomes *in vitro*, and variable effects in cells. It was originally identified alongside the 11S and 19S 'lid' activators in a screen for modulators of 20S proteasome activity [50]. It acted a potent competitor of PA28-mediated activation of the 20S proteasome in *in vitro* assays with proteasomes isolated from cells, by competitively



inhibiting the 19S activator [48]. In addition, it can also directly impact on proteasomal activity as evidenced by recombinant PI31 inhibiting 20S proteasome hydrolysis of peptides in similar assays [47]. These *in vitro* characterizations explain the naming of this protein as proteasome inhibitor 31kD. However, further experimentation in intact cells reveals a more complex story. In the yeast *S. cerevisiae*, the PI31 orthologue, Fub1 does not inhibit 20S proteasome activity, but instead appears to act as a positive regulator of proteasome assembly or activity, since it becomes essential for viability if the proteasome chaperone Pba4 is deleted [51]. Furthermore, the synthetic lethality of the double deletion of Fub1 and a Pba4, can be suppressed by deletion of the N-terminus of  $\beta 7$  subunit of the proteasome, which allows its partial activation. These findings indicate a positive regulatory role for Fub1 in enabling proteasome activity in yeast. No Fbxo7 homologues exist in yeast.

*Drosophila* encodes both a PI31 homologue (DmPI31) and an orthologue of Fbxo7, termed nutcracker. A major difference between the fly and mammalian Fbxo7 proteins is that nutcracker does not contain a C-terminal PRR, ending abruptly after its F-box domain, and the overall sequence homology between nutcracker and Fbxo7 is low (28% identity, 45% similarity). Moreover, human Fbxo7 cannot complement nutcracker function, as its expression does not rescue the sterility of nutcracker male flies [46]. That notwithstanding, there is strong evidence that DmPI31 and nutcracker show an interdependent regulation of proteasomal activity [52, 53]. Nutcracker is characterized as a protein that has grossly similar activities to its mammalian counterpart: it forms part of an SCF, interacts with an apoptosis-regulator called Bruce, binds DmPI31, and is required for male fly fertility. Interestingly, DmPI31 binds nutcracker via an interface that is also used by mammalian Fbxo7 and PI31 (see structures below). Nutcracker does not promote the ubiquitin-mediated degradation of DmPI31, but rather stabilizes DmPI31 levels and together their activities promote sperm production via the activation of caspases and an enhancement of proteasome activity [52, 53]. Thus, in contrast to its mammalian counterpart, DmPI31 activates 26S proteasomes *in vitro*, and like the yeast Fub1, DmPI31 also appears to enhance overall proteasome activity in cells [52].

Recently, a study of PI31 in human cells (HEK293 and HeLa cells) demonstrated no effects on overall proteasome content or activity upon its increased or decreased expression [49]. This may be due to the fact that the majority of ectopic PI31 in mammalian cells in this study was not found to be associated with the 26S proteasome [49]. It may be the case that PI31 is only utilized under specific conditions, in particular cell types (e.g. in testes in the adult fly), or is induced by explicit signaling to the proteasome. Support for this idea comes from a study by Zaiss, *et al.* who reported that PI31 overexpression in murine MEC cells also did not inhibit proteasome-mediated proteolysis [54]. Instead it interfered with the production and maturation of immunoproteasomes (ImP) in response to interferon  $\gamma$  (IFN $\gamma$ ) and consequently affected the processing and presentation of ImP-dependent epitopes on MHC class I [54]. The ImP is a variant of the constitutive proteasome, wherein the catalytic  $\beta$  subunits are replaced with IFN $\gamma$  inducible subunits,  $\beta 1i$  (LMP2),  $\beta 2i$  (MECL1), and  $\beta 5i$  (LMP7), and the 19S 'lid' is replaced with an 11S 'lid' made from PA28  $\alpha$  and  $\beta$  subunits [55]. These subunit replacements confer altered cleavage specificities to the ImP and also supra-normal activity that boosts proteolysis under stress conditions, including viral infection and oxidative or metabolic stress [56]. In the context of neurodegenerative disease, the ImP may have particular relevance as it is selectively able to degrade oxidized proteins [56, 57], and an increased capacity to degrade damaged proteins may diminish the formation of protein aggregates [52].

### **3D structures of the FP domains of Fbxo7 and PI31**

The structures for the homologous FP domains of both Fbxo7 and PI31 have been solved [39, 58, 59]. This domain appears only within these two proteins, and no other solved protein structures appear to have their particular topology. The FP domain of PI31 is approximately 40 x 28 x 25 Å in size and consists of an  $\alpha/\beta$ -fold with a central five-stranded anti-parallel  $\beta$ -sheet flanked by two N-terminal  $\alpha$ -helices and three C-terminal  $\alpha$ -helices. The  $\alpha$ -helices pack against one face of the  $\beta$ -sheet, like a fist against a palm, leaving the  $\beta$ -sheet face openly accessible [39]. Gel filtration of cell lysates indicate that the PI31 FP protomer exists predominantly in a dimeric state. Its homo-dimerization takes place through residues in the first  $\alpha$ -helix, and this is supported by the fact that mutation of Val6 in helix  $\alpha$ 1 prevents this. There is a hydrophobic patch located on the surface of the  $\beta$ -sheet, consisting of residues L64, I83, V85, and I90, which play a critical role in mediating protein-protein interactions between mammalian and *Drosophila* PI31 and Fbxo7 [39, 52]. Point mutations in PI31 at I83 and I90 are sufficient to abrogate their hetero-dimerization. The residue equivalent to I83 of PI31's FP domain within the  $\beta$ -sheet of Fbxo7's FP domain is V253. When this amino acid is mutated, it prevents Fbxo7 hetero-dimerization with PI31 and its own homo-dimerization, indicating that the larger  $\beta$ -sheet surface is used for Fbxo7 dimerizing protein-protein interactions [39].

Recently, the crystal structure of the FP domain within Fbxo7 was reported (Figure 1C-i), and it bears high structural similarity with a comparable overall fold to that of PI31 [58, 59]. Two additional well-structured loops in Fbxo7 were described: one of 9 amino acids in length between  $\alpha$  helices  $\alpha$ 3 and  $\alpha$ 4, and one of 17 amino acids between  $\beta$ -strands  $\beta$ 1 and  $\beta$ 2. The C-terminal end of the FP domain in Fbxo7, comprising helix  $\alpha$ 5 and strands  $\beta$ 3,  $\beta$ 4,  $\beta$ 5, is longer and more extended, giving more structure to this region. The striking hydrophobic patch observed in the FP domain of PI31 which mediated its hetero-dimerization with Fbxo7 appears conserved within the FP domain of Fbxo7. Most of the residues of helix  $\alpha$ 2 of Fbxo7 are hydrophobic, including 11 amino acids starting at amino acid 198 (ALIVLIHLLML). These amino acids, along with multiple others comprise its hydrophobic core, which gives it a strong, tightly packed structural stability. This compact, stable structure contributes to the two conformations adopted for inter-molecular interactions of Fbxo7's FP protomer, described as an ' $\alpha\beta$  interface' due to the fact that  $\alpha$ -helices in one protomer and  $\beta$ -strands in the other protomer are proposed to mediate interactions (Figure 1C-ii) [58]. This contrasts the exclusively  $\alpha$ -helical interface for homo-dimerization or  $\beta$ -sheet interactions for hetero-dimerizing interactions of the PI31 FP protomer [39]. The FP domain of Fbxo7 was predicted to be dimeric, and this is suggested to be due to the capacity of the FP domain to interact with adjacent FP domains using either the  $\alpha$ -helical or  $\beta$ -sheet interacting surfaces located on opposing sides of the protomer. This capacity for dual use of the ' $\alpha\beta$  interface' implies strongly that Fbxo7 homo-dimerizes, a common strategy for SCF-type ubiquitin ligases to regulate their activity, although this has not yet been tested for Fbxo7 in cells. What's more, these studies raise the possibility for multimerization of Fbxo7, with or without PI31, in extended 'hand-holding' conformations. It has been recently suggested that such a hetero-dimer could form via an  $\alpha/\beta$  interaction of the  $\alpha$ -helical surface of Fbxo7 and the  $\beta$ -sheet surfaces of PI31 FP domains [58], but this has not been directly tested.

In deducing the interacting interfaces for PI31 and Fbxo7, Kirk, *et al.* reported that PI31 can interact with Fbxo7 in the presence of Skp1, yet PI31 is not ubiquitinated [39].

Similarly Bader, *et al.* reported that nutcracker does not ubiquitinate DmPI31 [52]. These findings leave open the possibility that PI31 could impact on SCF<sup>Fbxo7</sup> activity, potentially determining substrate choice or ubiquitin-chain linkage types, or on the non-canonical roles of Fbxo7. Alternatively or additionally, the high affinity interaction between mammalian Fbxo7 and PI31 suggests that Fbxo7 may stabilize PI31 levels, as is the case in flies, and/or affect PI31's function regarding proteasomal regulation. However, any connection between PI31 and Fbxo7 in regulating mammalian proteasomal activity has not yet been reported. In either scenario it must also be appreciated that at least in cultured mammalian cells, the majority of PI31 and Fbxo7 appear in different subcellular locations. Endogenous PI31 has been shown to localize to the endoplasmic reticulum, while Fbxo7 shuttles between the nucleus and cytoplasm in a cell cycle-dependent fashion [37, 39, 54]. Any co-regulation by PI31 and Fbxo7 of proteasomal function may be spatially or temporally restricted to specialized cell types or particular circumstances that require high levels of proteasome activity. Since UPS dysfunction and protein aggregation are suspected factors in PD etiology, it is tempting to speculate that Fbxo7 regulation of the proteasome, possibly via its interaction with PI31, might also play a part in this disease. However, no mutations have been mapped within the dimerizing FP domain, although a p.D328R substitution which lies just beyond helix  $\alpha$ 5b and before the start of the NES/F-box domain has been recently found [16]. It remains to be seen if this affects dimerization, localization or ubiquitin ligase activity.

### **The Ubl domain of Fbxo7**

Fbxo7 is the only F-box protein among the Fbxo subfamily to contain at its N-terminus a Ubl domain. This domain is present only in its longest and most abundant isoform 1, which is 522 amino acids in length. Its second isoform, of 443 amino acids, is encoded from an alternative 5' exon and skips the exon which encodes the Ubl domain [42]. Ubl domains have both structural and sequence homology to ubiquitin, which is among the most highly conserved proteins. Of its 76 amino acids, only three residues diverge from mammals to plants and yeasts. Similar to the FP domain, ubiquitin forms a five strand  $\beta$ -sheet, but it has only a single  $\alpha$ -helix atop. Proteins which contain a Ubl domain are numerous and fall into two categories: those which are used in a fashion similar to ubiquitin such as SUMO, i.e. added as a modification and used in cell signaling, albeit distinct from ubiquitin itself, and those which do not act as protein modifiers, but instead incorporate this domain as an integral part of their sequence.

A generalized function for the Ubl domain among this second class of proteins is that they have the capacity to bind to the S5a/RPN10/PSMD4 subunit of 26S proteasomes. However, these Ubl domain-containing proteins escape proteasomal degradation [60, 61]. HHR23A/Rad23A is, for example, a well-characterized protein that contains a Ubl domain (the crystal structure for which is shown in Figure 1B) and transports poly-ubiquitinated proteins to the proteasome for their subsequent destruction [62, 63]. On the proteasome and within the lid, the S5a subunit is essential for this efficient degradation of poly-ubiquitinated proteins via the recruitment of Ubl-domain containing proteins, which are recognized by the ubiquitin-interacting motif (UIM) in S5a. In addition, a second VWA motif within S5a restricts these UIM interactions from occurring except when it is associated with the 26S proteasome [64]. Fbxo7 falls into this latter category of Ubl-containing proteins, and can interact directly with the S5a subunit (our unpublished data). One immediate prediction

of their association is that it would link ubiquitination of SCF<sup>Fbxo7</sup> substrates directly to the proteasome to promote their degradation.

Structural insight into the interaction of a Ubl domain within HHR23A to S5a has been undertaken for both the full length protein [65], and an isolated UIM domain [66]. In the Ubl domain, multiple residues within the five-stranded  $\beta$ -sheet (I9, L10, K47, L48, I49, K53, I54, L55, V73, M75), its connecting loops (E11, E12, G45, A51, S56, V59), and a C-terminal residue (K78) are proposed to be important contributors to UIM binding. Among these is K53, the equivalent residue to K48 in ubiquitin that is important for poly-ubiquitin chain linkages [66]. Interestingly, this K53 is conserved within the Ubl of Fbxo7, and is near the Y52 residue which appears as a potential protective allele of Fbxo7 when substituted with cysteine. One possibility is that a p.Y52C mutation may affect Fbxo7 affinity for S5a and its interaction with the proteasome. By comparison, Parkin, like Fbxo7, is an ubiquitin ligase and contains an N-terminal Ubl domain that binds to S5a. The full length structure of Parkin has recently been solved [67, 68] and is discussed in detail elsewhere in this issue. One of the pathogenic mutations within the Ubl domain of Parkin (p.R42P) has been suggested to impair its interaction with the proteasome and this may contribute to Parkin-mediated pathology [69]. The effect of a p.Y52C substitution on Fbxo7 protein levels and/or on the SCF<sup>Fbxo7</sup> ubiquinome remains to be determined.

A definitive role for the Ubl domain of Fbxo7 in direct interactions with both Parkin and PINK1 has recently been published. Burchell, *et al.* showed that Fbxo7 is recruited to mitochondria and plays a role in the PINK1/Parkin-regulated pathway for autophagic clearance of damaged or depolarized mitochondria [46]. The relationship between PINK1/Parkin and their control of mitophagy has been well documented and is also detailed in other articles in this issue. In brief, the current model is that PINK1 is activated by the loss of inner mitochondrial membrane potential which promotes its auto-phosphorylation and subsequent phosphorylation of Parkin on S65, within its N-terminal Ubl domain, which activates its E3 ubiquitin ligase activity and primes its mitochondrial translocation [70-73]. Parkin's Ubl domain has been demonstrated to restrain its intrinsic auto-ubiquitination capacity through an intramolecular association with its C-terminus, indicating an auto-inhibitory mechanism. Moreover, pathogenic missense mutations in Parkin including p.R42P and p.K48A disrupt this auto-inhibition, suggesting this interaction is important in PD [74]. Although the mechanism by which Fbxo7 participates in mitophagy remains to be determined, Burchell, *et al.* proposed that Fbxo7 may act as a scaffold to facilitate PINK1-mediated phosphorylation and activation of Parkin, and also ubiquitinate its own specific, but as yet unknown, targets to promote mitophagy [46]. A pathogenic mutation, p.T22M within the Ubl interferes with Parkin binding, recruitment and subsequent mitophagy, and a p.T22M allele of Fbxo7 cannot rescue the mitophagy defect seen with knockdown of WT Fbxo7. The PINK1 binding site on Fbxo7 includes the Ubl domain and extends further along the protein, but its binding is not affected by the p.T22M mutation [46]. Interestingly, fibroblasts from patients with compound heterozygous p.T22M and IVS7 + 1G/T mutations show an absence of Fbxo7 isoform 1 expression, but not isoform 2, suggesting these mutations may also destabilize the protein, at least in fibroblasts [45]. It is tempting to speculate that given their similar enzymatic activities and Ubl domains at the N-terminus that Fbxo7 may, like Parkin, have an intramolecular interaction involving its Ubl domain that regulates its auto-ubiquitination and/or its ubiquitin ligase activity. Indeed the critical serine residue within ubiquitin and Parkin that is phosphorylated by PINK1 is present within Fbxo7. However, in a screen for substrates, Fbxo7 was not phosphorylated by PINK1 [71].

Nevertheless, Fbxo7 was found to function downstream of PINK1 as Fbxo7 relocation was PINK1-dependent. Also the expression of Fbxo7 could not rescue the phenotypes associated with PINK1 silencing in mammalian cells or in *Drosophila*. The mechanistic basis for this is as yet unknown.

In addition to mitophagy regulation, another potential function for the Ubl domain in Fbxo7 may lay in the fact that the Ubl domains can aid in resolving or clearing protein aggregates. Evidence for this capacity for Ubl domains comes from a critical Ubl-UIM interaction between PLIC-1 and EPS15, AT3 and HSI1a, which are known to be involved in the clearance of misfolded and aggregated proteins [75]. The presence of Fbxo7 in Lewy Bodies in idiopathic PD brain samples suggests they play some role at these sites of protein deposition. The capacity for multiple interactions of the Ubl domain affecting two suspect pathogenic pathways in PD may explain Fbxo7's involvement in this disease.

### **Concluding remarks**

Fbxo7/PARK15 is a highly engineered, multi-functional protein with effects on multiple cellular pathways, including NF- $\kappa$ B and NGF signaling, cell cycle, proteasome regulation, and mitophagy, and with differential, sometimes contradictory, effects in specific cell types. This diversity of functions is likely to reflect its capacity to work as a networking hub that integrates cellular responses, either through its activities as an E3 ligase or as a scaffolding platform. The identification of pathogenic mutations within its specific functional domains helps focus researchers on particular activities; however, it will undoubtedly be necessary to investigate their consequences in the relevant cell type and physiological setting(s) to understand their impact across the network of processes that Fbxo7 regulates. Fbxo7 regulation of multiple pathways implicated in the etiology of PD may explain why it is a PARK gene. Our challenge now is to find out the mechanism(s) and to exploit this understanding to benefit patients.

## Acknowledgements

This work is funded by the BBSRC (BB/J007846/1).

## References

- [1] Dorsey, E. R.; Constantinescu, R.; Thompson, J. P.; Biglan, K. M.; Holloway, R. G.; Kieburtz, K.; Marshall, F. J.; Ravina, B. M.; Schifitto, G.; Siderowf, A.; Tanner, C. M., Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*, **2007**, *68* (5), 384-6.
- [2] Taylor, K. S.; Cook, J. A.; Counsell, C. E., Heterogeneity in male to female risk for Parkinson's disease. *J Neurol Neurosurg Psychiatry*, **2007**, *78* (8), 905-6.
- [3] Burn, D. J., Sex and Parkinson's disease: a world of difference? *J Neurol Neurosurg Psychiatry*, **2007**, *78* (8), 787.
- [4] Moore, D. J.; West, A. B.; Dawson, V. L.; Dawson, T. M., Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci*, **2005**, *28*, 57-87.
- [5] Belin, A. C.; Westerlund, M., Parkinson's disease: a genetic perspective. *FEBS J*, **2008**, *275* (7), 1377-83.
- [6] Davison, C., Pallido-pyramidal disease. *J Neuropathol Exp Neurol*, **1954**, *13* (1), 50-9.
- [7] Horstink, M. W.; Dekker, M. C.; Montagna, P.; Bonifati, V.; van De Warrenburg, B. P., Pallidopyramidal disease: a misnomer? *Mov Disord*, **2010**, *25* (9), 1109-15.
- [8] Deng, H.; Liang, H.; Jankovic, J., F-box only protein 7 gene in parkinsonian-pyramidal disease. *JAMA Neurol*, **2013**, *70* (1), 20-4.
- [9] Zhao, T.; Severijnen, L. A.; van der Weiden, M.; Zheng, P. P.; Oostra, B. A.; Hukema, R. K.; Willemsen, R.; Kros, J. M.; Bonifati, V., FBXO7 immunoreactivity in alpha-synuclein-containing inclusions in Parkinson disease and multiple system atrophy. *J Neuropathol Exp Neurol*, **2013**, *72* (6), 482-8.
- [10] Di Fonzo, A.; Dekker, M. C.; Montagna, P.; Baruzzi, A.; Yonova, E. H.; Correia Guedes, L.; Szczerbinska, A.; Zhao, T.; Dubbel-Hulsman, L. O.; Wouters, C. H.; de Graaff, E.; Oyen, W. J.; Simons, E. J.; Breedveld, G. J.; Oostra, B. A.; Horstink, M. W.; Bonifati, V., FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. *Neurology*, **2009**, *72* (3), 240-5.
- [11] Paisan-Ruiz, C.; Guevara, R.; Federoff, M.; Hanagasi, H.; Sina, F.; Elahi, E.; Schneider, S. A.; Schwingenschuh, P.; Bajaj, N.; Emre, M.; Singleton, A. B.; Hardy, J.; Bhatia, K. P.; Brandner, S.; Lees, A. J.; Houlden, H., Early-onset L-dopa-responsive parkinsonism with pyramidal signs due to ATP13A2, PLA2G6, FBXO7 and spatacsin mutations. *Mov Disord*, **2010**, *25* (12), 1791-800.
- [12] Shojaee, S.; Sina, F.; Banihosseini, S. S.; Kazemi, M. H.; Kalhor, R.; Shahidi, G. A.; Fakhrai-Rad, H.; Ronaghi, M.; Elahi, E., Genome-wide linkage analysis of a Parkinsonian-pyramidal syndrome pedigree by 500 K SNP arrays. *Am J Hum Genet*, **2008**, *82* (6), 1375-84.
- [13] Gunduz, A.; Eken, A. G.; Bilgic, B.; Hanagasi, H. A.; Bilguvar, K.; Gunel, M.; Basak, A. N.; Ertan, S., FBXO7-R498X mutation: Phenotypic variability from chorea to early onset parkinsonism within a family. *Parkinsonism Relat Disord*, **2014**.
- [14] Yalcin-Cakmakli, G.; Olgiati, S.; Quadri, M.; Breedveld, G. J.; Cortelli, P.; Bonifati, V.; Elibol, B., A new Turkish family with homozygous FBXO7 truncating mutation and juvenile atypical parkinsonism. *Parkinsonism Relat Disord*, **2014**.
- [15] Santoro, L.; Breedveld, G. J.; Manganelli, F.; Iodice, R.; Pisciotta, C.; Nolano, M.; Punzo, F.; Quarantelli, M.; Pappata, S.; Di Fonzo, A.; Oostra, B. A.; Bonifati, V., Novel ATP13A2 (PARK9) homozygous mutation in a family with marked phenotype variability. *Neurogenetics*, **2011**, *12* (1), 33-9.
- [16] Lin, C. H.; Chen, M. L.; Lai, T. T.; Tai, C. H.; Wu, R. M., Mutational analysis of FBXO7 gene in Parkinson's disease in a Taiwanese population. *Neurobiol Aging*, **2013**, *34* (6), 1713 e1-4.

- [17] Chen, C. M.; Chen, I. C.; Huang, Y. C.; Juan, H. F.; Chen, Y. L.; Chen, Y. C.; Lin, C. H.; Lee, L. C.; Lee, C. M.; Lee-Chen, G. J.; Lai, Y. J.; Wu, Y. R., FBXO7 Y52C polymorphism as a potential protective factor in Parkinson's disease. *PLoS One*, **2014**, *9* (7), e101392.
- [18] Ding, K.; Shameer, K.; Jouni, H.; Masys, D. R.; Jarvik, G. P.; Kho, A. N.; Ritchie, M. D.; McCarty, C. A.; Chute, C. G.; Manolio, T. A.; Kullo, I. J., Genetic Loci implicated in erythroid differentiation and cell cycle regulation are associated with red blood cell traits. *Mayo Clin Proc*, **2012**, *87* (5), 461-74.
- [19] Zhu, L. H.; Luo, X. G.; Zhou, Y. S.; Li, F. R.; Yang, Y. C.; Ren, Y.; Pang, H., Lack of association between three single nucleotide polymorphisms in the PARK9, PARK15, and BST1 genes and Parkinson's disease in the northern Han Chinese population. *Chin Med J (Engl)*, **2012**, *125* (4), 588-92.
- [20] Laman, H.; Funes, J. M.; Ye, H.; Henderson, S.; Galinanes-Garcia, L.; Hara, E.; Knowles, P.; McDonald, N.; Boshoff, C., Transforming activity of Fbxo7 is mediated specifically through regulation of cyclin D/cdk6. *EMBO J*, **2005**, *24* (17), 3104-16.
- [21] Winston, J. T.; Koepf, D. M.; Zhu, C.; Elledge, S. J.; Harper, J. W., A family of mammalian F-box proteins. *Curr Biol*, **1999**, *9* (20), 1180-2.
- [22] Cenciarelli, C.; Chiaur, D. S.; Guardavaccaro, D.; Parks, W.; Vidal, M.; Pagano, M., Identification of a family of human F-box proteins. *Curr Biol*, **1999**, *9* (20), 1177-9.
- [23] Zheng, N.; Schulman, B. A.; Song, L.; Miller, J. J.; Jeffrey, P. D.; Wang, P.; Chu, C.; Koepf, D. M.; Elledge, S. J.; Pagano, M.; Conaway, R. C.; Conaway, J. W.; Harper, J. W.; Pavletich, N. P., Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. *Nature*, **2002**, *416* (6882), 703-9.
- [24] Lee, J. E.; Sweredoski, M. J.; Graham, R. L.; Kolawa, N. J.; Smith, G. T.; Hess, S.; Deshaies, R. J., The steady-state repertoire of human SCF ubiquitin ligase complexes does not require ongoing Nedd8 conjugation. *Mol Cell Proteomics*, **2011**, *10* (5), M110 006460.
- [25] Hsu, J. M.; Lee, Y. C.; Yu, C. T.; Huang, C. Y., Fbx7 functions in the SCF complex regulating Cdk1-cyclin B-phosphorylated hepatoma up-regulated protein (HURP) proteolysis by a proline-rich region. *J Biol Chem*, **2004**, *279* (31), 32592-602.
- [26] Chang, Y. F.; Cheng, C. M.; Chang, L. K.; Jong, Y. J.; Yuo, C. Y., The F-box protein Fbxo7 interacts with human inhibitor of apoptosis protein cIAP1 and promotes cIAP1 ubiquitination. *Biochem Biophys Res Commun*, **2006**, *342* (4), 1022-6.
- [27] Kuiken, H. J.; Egan, D. A.; Laman, H.; Bernards, R.; Beijersbergen, R. L.; Dirac, A. M., Identification of F-box only protein 7 as a negative regulator of NF-kappaB signalling. *J Cell Mol Med*, **2012**, *16* (9), 2140-9.
- [28] Kang, J.; Chung, K. C., The F-box protein FBXO7 positively regulates bone morphogenetic protein-mediated signaling through Lys-63-specific ubiquitination of neurotrophin receptor-interacting MAGE (NRAGE). *Cell Mol Life Sci*, **2014**.
- [29] Ye, F.; Tan, L.; Yang, Q.; Xia, Y.; Deng, L. W.; Murata-Hori, M.; Liou, Y. C., HURP regulates chromosome congression by modulating kinesin Kif18A function. *Curr Biol*, **2011**, *21* (18), 1584-91.
- [30] Tsou, A. P.; Yang, C. W.; Huang, C. Y.; Yu, R. C.; Lee, Y. C.; Chang, C. W.; Chen, B. R.; Chung, Y. F.; Fann, M. J.; Chi, C. W.; Chiu, J. H.; Chou, C. K., Identification of a novel cell cycle regulated gene, HURP, overexpressed in human hepatocellular carcinoma. *Oncogene*, **2003**, *22* (2), 298-307.
- [31] Chen, Z. J.; Sun, L. J., Nonproteolytic functions of ubiquitin in cell signaling. *Mol Cell*, **2009**, *33* (3), 275-86.
- [32] Nathan, J. A.; Kim, H. T.; Ting, L.; Gygi, S. P.; Goldberg, A. L., Why do cellular proteins linked to K63-polyubiquitin chains not associate with proteasomes? *EMBO J*, **2013**, *32* (4), 552-65.
- [33] Tan, J. M.; Wong, E. S.; Kirkpatrick, D. S.; Pletnikova, O.; Ko, H. S.; Tay, S. P.; Ho, M. W.; Troncoso, J.; Gygi, S. P.; Lee, M. K.; Dawson, V. L.; Dawson, T. M.; Lim, K. L., Lysine 63-linked ubiquitination promotes the formation and autophagic clearance of protein inclusions associated with neurodegenerative diseases. *Hum Mol Genet*, **2008**, *17* (3), 431-9.
- [34] Skaug, B.; Jiang, X.; Chen, Z. J., The role of ubiquitin in NF-kappaB regulatory pathways. *Annu Rev Biochem*, **2009**, *78*, 769-96.

- [35] Bertrand, M. J.; Milutinovic, S.; Dickson, K. M.; Ho, W. C.; Boudreault, A.; Durkin, J.; Gillard, J. W.; Jaquith, J. B.; Morris, S. J.; Barker, P. A., cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. *Mol Cell*, **2008**, *30* (6), 689-700.
- [36] Mahoney, D. J.; Cheung, H. H.; Mrad, R. L.; Plenchette, S.; Simard, C.; Enwere, E.; Arora, V.; Mak, T. W.; Lacasse, E. C.; Waring, J.; Korneluk, R. G., Both cIAP1 and cIAP2 regulate TNF $\alpha$ -mediated NF- $\kappa$ B activation. *Proc Natl Acad Sci U S A*, **2008**, *105* (33), 11778-83.
- [37] Nelson, D. E.; Laman, H., A Competitive binding mechanism between Skp1 and exportin 1 (CRM1) controls the localization of a subset of F-box proteins. *J Biol Chem*, **2011**, *286* (22), 19804-15.
- [38] Morgan, A. A.; Rubenstein, E., Proline: the distribution, frequency, positioning, and common functional roles of proline and polyproline sequences in the human proteome. *PLoS One*, **2013**, *8* (1), e53785.
- [39] Kirk, R.; Laman, H.; Knowles, P. P.; Murray-Rust, J.; Lomonosov, M.; Meziane el, K.; McDonald, N. Q., Structure of a conserved dimerization domain within the F-box protein Fbxo7 and the PI31 proteasome inhibitor. *J Biol Chem*, **2008**, *283* (32), 22325-35.
- [40] Sherr, C. J.; Roberts, J. M., CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev*, **1999**, *13* (12), 1501-12.
- [41] Meziane, e. K.; Randle, S. J.; Nelson, D. E.; Lomonosov, M.; Laman, H., Knockdown of Fbxo7 reveals its regulatory role in proliferation and differentiation of haematopoietic precursor cells. *J Cell Sci*, **2011**, *124* (Pt 13), 2175-86.
- [42] Nelson, D. E.; Randle, S. J.; Laman, H., Beyond ubiquitination: the atypical functions of Fbxo7 and other F-box proteins. *Open Biol*, **2013**, *3* (10), 130131.
- [43] Beukelaers, P.; Vandebosch, R.; Caron, N.; Nguyen, L.; Belachew, S.; Moonen, G.; Kiyokawa, H.; Barbacid, M.; Santamaria, D.; Malgrange, B., Cdk6-dependent regulation of G(1) length controls adult neurogenesis. *Stem Cells*, **2011**, *29* (4), 713-24.
- [44] Andreu, Z.; Khan, M. A.; Gonzalez-Gomez, P.; Negueruela, S.; Hortiguuela, R.; San Emeterio, J.; Ferron, S. R.; Martinez, G.; Vidal, A.; Farinas, I.; Lie, D. C.; Mira, H., The cyclin-dependent kinase inhibitor p27 regulates radial stem cell quiescence and neurogenesis in the adult hippocampus. *Stem Cells*, **2014**.
- [45] Zhao, T.; De Graaff, E.; Breedveld, G. J.; Loda, A.; Severijnen, L. A.; Wouters, C. H.; Verheijen, F. W.; Dekker, M. C.; Montagna, P.; Willemsen, R.; Oostra, B. A.; Bonifati, V., Loss of nuclear activity of the FBXO7 protein in patients with parkinsonian-pyramidal syndrome (PARK15). *PLoS One*, **2011**, *6* (2), e16983.
- [46] Burchell, V. S.; Nelson, D. E.; Sanchez-Martinez, A.; Delgado-Camprubi, M.; Ivatt, R. M.; Pogson, J. H.; Randle, S. J.; Wray, S.; Lewis, P. A.; Houlden, H.; Abramov, A. Y.; Hardy, J.; Wood, N. W.; Whitworth, A. J.; Laman, H.; Plun-Favreau, H., The Parkinson's disease-linked proteins Fbxo7 and Parkin interact to mediate mitophagy. *Nat Neurosci*, **2013**, *16* (9), 1257-65.
- [47] McCutchen-Maloney, S. L.; Matsuda, K.; Shimbara, N.; Binns, D. D.; Tanaka, K.; Slaughter, C. A.; DeMartino, G. N., cDNA cloning, expression, and functional characterization of PI31, a proline-rich inhibitor of the proteasome. *J Biol Chem*, **2000**, *275* (24), 18557-65.
- [48] Zaiss, D. M.; Standera, S.; Holzhtutter, H.; Kloetzel, P.; Sijts, A. J., The proteasome inhibitor PI31 competes with PA28 for binding to 20S proteasomes. *FEBS Lett*, **1999**, *457* (3), 333-8.
- [49] Li, X.; Thompson, D.; Kumar, B.; DeMartino, G. N., Molecular and cellular roles of PI31 (PSMF1) protein in regulation of proteasome function. *J Biol Chem*, **2014**, *289* (25), 17392-405.
- [50] Chu-Ping, M.; Vu, J. H.; Proske, R. J.; Slaughter, C. A.; DeMartino, G. N., Identification, purification, and characterization of a high molecular weight, ATP-dependent activator (PA700) of the 20 S proteasome. *J Biol Chem*, **1994**, *269* (5), 3539-47.
- [51] Yashiroda, H.; Toda, Y.; Otsu, S.; Takagi, K.; Mizushima, T.; Murata, S., N-terminal alpha7 deletion of the proteasome 20S core particle substitutes for yeast PI31 function. *Mol Cell Biol*, **2014**.
- [52] Bader, M.; Benjamin, S.; Wapinski, O. L.; Smith, D. M.; Goldberg, A. L.; Steller, H., A conserved F box regulatory complex controls proteasome activity in Drosophila. *Cell*, **2011**, *145* (3), 371-82.

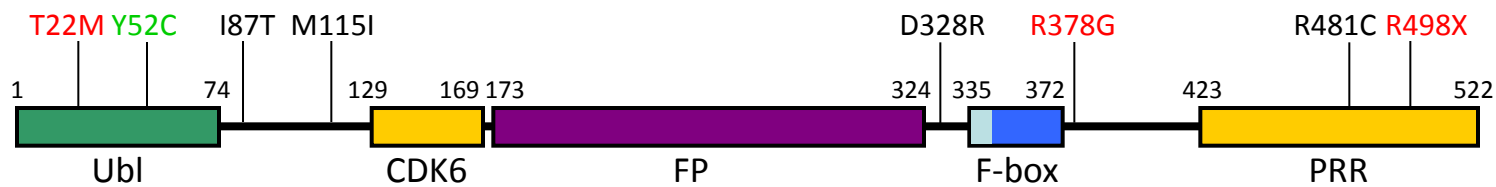
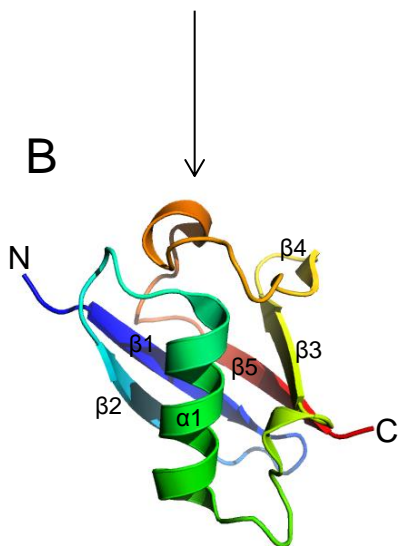
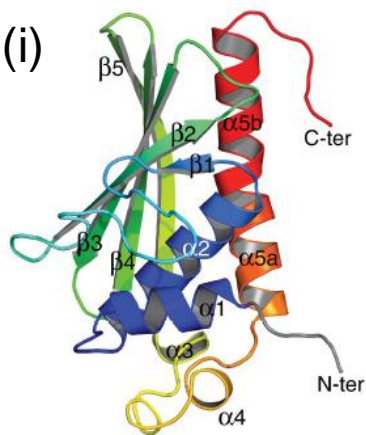
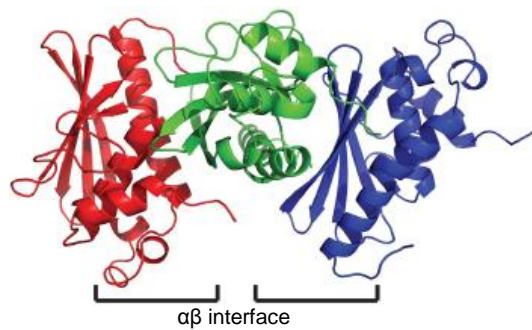


- [53] Bader, M.; Arama, E.; Steller, H., A novel F-box protein is required for caspase activation during cellular remodeling in *Drosophila*. *Development*, **2010**, *137* (10), 1679-88.
- [54] Zaiss, D. M.; Standera, S.; Kloetzel, P. M.; Sijts, A. J., PI31 is a modulator of proteasome formation and antigen processing. *Proc Natl Acad Sci U S A*, **2002**, *99* (22), 14344-9.
- [55] Rechsteiner, M.; Realini, C.; Ustrell, V., The proteasome activator 11 S REG (PA28) and class I antigen presentation. *Biochem J*, **2000**, *345 Pt 1*, 1-15.
- [56] Bellavista, E.; Andreoli, F.; Parenti, M. D.; Martucci, M.; Santoro, A.; Salvioli, S.; Capri, M.; Baruzzi, A.; Del Rio, A.; Franceschi, C.; Mishto, M., Immunoproteasome in cancer and neuropathologies: a new therapeutic target? *Curr Pharm Des*, **2013**, *19* (4), 702-18.
- [57] Pickering, A. M.; Koop, A. L.; Teoh, C. Y.; Ermak, G.; Grune, T.; Davies, K. J., The immunoproteasome, the 20S proteasome and the PA28alpha proteasome regulator are oxidative-stress-adaptive proteolytic complexes. *Biochem J*, **2010**, *432* (3), 585-94.
- [58] Shang, J.; Huang, X.; Du, Z., The FP domains of PI31 and Fbxo7 have the same protein fold but very different modes of protein-protein interaction. *J Biomol Struct Dyn*, **2014**, 1-11.
- [59] Shang, J.; Wang, G.; Yang, Y.; Huang, X.; Du, Z., Structure of the FP domain of Fbxo7 reveals a novel mode of protein-protein interaction. *Acta Crystallogr D Biol Crystallogr*, **2014**, *70* (Pt 1), 155-64.
- [60] Wilkinson, C. R., Ubiquitin-like proteins: meet the family. *Seminars in cell & developmental biology*, **2004**, *15* (2), 199-200.
- [61] Hartmann-Petersen, R.; Gordon, C., Integral UBL domain proteins: a family of proteasome interacting proteins. *Seminars in cell & developmental biology*, **2004**, *15* (2), 247-59.
- [62] Liang, R. Y.; Chen, L.; Ko, B. T.; Shen, Y. H.; Li, Y. T.; Chen, B. R.; Lin, K. T.; Madura, K.; Chuang, S. M., Rad23 interaction with the proteasome is regulated by phosphorylation of its ubiquitin-like (Ubl) domain. *J Mol Biol*, **2014**.
- [63] Schaubert, C.; Chen, L.; Tongaonkar, P.; Vega, I.; Lambertson, D.; Potts, W.; Madura, K., Rad23 links DNA repair to the ubiquitin/proteasome pathway. *Nature*, **1998**, *391* (6668), 715-8.
- [64] Piterman, R.; Braunstein, I.; Isakov, E.; Ziv, T.; Navon, A.; Cohen, S.; Stanhill, A., Vwa Domain of S5a Restricts the Ability to Bind Ubiquitin and Ubl to the 26s Proteasome. *Mol Biol Cell*, **2014**.
- [65] Walters, K. J.; Kleijnen, M. F.; Goh, A. M.; Wagner, G.; Howley, P. M., Structural studies of the interaction between ubiquitin family proteins and proteasome subunit S5a. *Biochemistry*, **2002**, *41* (6), 1767-77.
- [66] Mueller, T. D.; Feigon, J., Structural determinants for the binding of ubiquitin-like domains to the proteasome. *EMBO J*, **2003**, *22* (18), 4634-45.
- [67] Wauer, T.; Komander, D., Structure of the human Parkin ligase domain in an autoinhibited state. *EMBO J*, **2013**, *32* (15), 2099-112.
- [68] Trempe, J. F.; Sauve, V.; Grenier, K.; Seirafi, M.; Tang, M. Y.; Menade, M.; Al-Abdul-Wahid, S.; Krett, J.; Wong, K.; Kozlov, G.; Nagar, B.; Fon, E. A.; Gehring, K., Structure of parkin reveals mechanisms for ubiquitin ligase activation. *Science*, **2013**, *340* (6139), 1451-5.
- [69] Sakata, E.; Yamaguchi, Y.; Kurimoto, E.; Kikuchi, J.; Yokoyama, S.; Yamada, S.; Kawahara, H.; Yokosawa, H.; Hattori, N.; Mizuno, Y.; Tanaka, K.; Kato, K., Parkin binds the Rpn10 subunit of 26S proteasomes through its ubiquitin-like domain. *EMBO Rep*, **2003**, *4* (3), 301-6.
- [70] Kazlauskaitė, A.; Kondapalli, C.; Gourlay, R.; Campbell, D. G.; Ritorto, M. S.; Hofmann, K.; Alessi, D. R.; Knebel, A.; Trost, M.; Muqit, M. M., Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem J*, **2014**, *460* (1), 127-39.
- [71] Kondapalli, C.; Kazlauskaitė, A.; Zhang, N.; Woodroof, H. I.; Campbell, D. G.; Gourlay, R.; Burchell, L.; Walden, H.; Macartney, T. J.; Deak, M.; Knebel, A.; Alessi, D. R.; Muqit, M. M., PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol*, **2012**, *2* (5), 120080.
- [72] Shiba-Fukushima, K.; Imai, Y.; Yoshida, S.; Ishihama, Y.; Kanao, T.; Sato, S.; Hattori, N., PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes mitochondrial translocation of Parkin and regulates mitophagy. *Scientific reports*, **2012**, *2*, 1002.

- [73] Caulfield, T. R.; Fiesel, F. C.; Moussaud-Lamodiere, E. L.; Dourado, D. F.; Flores, S. C.; Springer, W., Phosphorylation by PINK1 Releases the UBL Domain and Initializes the Conformational Opening of the E3 Ubiquitin Ligase Parkin. *PLoS Comput Biol*, **2014**, *10* (11), e1003935.
- [74] Chaugule, V. K.; Burchell, L.; Barber, K. R.; Sidhu, A.; Leslie, S. J.; Shaw, G. S.; Walden, H., Autoregulation of Parkin activity through its ubiquitin-like domain. *EMBO J*, **2011**, *30* (14), 2853-67.
- [75] Heir, R.; Ablasou, C.; Dumontier, E.; Elliott, M.; Fagotto-Kaufmann, C.; Bedford, F. K., The UBL domain of PLIC-1 regulates aggresome formation. *EMBO Rep*, **2006**, *7* (12), 1252-8.
- [76] Chen, Y. W.; Tajima, T.; Agrawal, S., The crystal structure of the ubiquitin-like (Ubl) domain of human homologue A of Rad23 (hHR23A) protein. *Protein Eng Des Sel*, **2010**, *24* (1-2), 131-8.

Figure 1. Human Fbxo7 protein structure. (A) Fbxo7 isoform 1 protein structure with structural domains, and clinically relevant SNPs highlighted above. A nuclear export signal within the F-box domain is in a lighter shade. Familial pathogenic PD mutations are T22M, R378G and R498X, a protective SNP is Y52C, and other SNPs identified in idiopathic PD patients (I87T, D328R and R481C) or associated with erythroid parameters (M115I) are also shown. (B-D) Exemplar crystal structures for domains within Fbxo7 are shown, with the N and C terminal labeled and  $\alpha$ -helices and  $\beta$ -strands numbered in order. (B) Cartoon of the ubiquitin-like (Ubl) domain of hHR23A, as determined by X-ray crystallography (PDB ID: 2WYQ). Reprinted with permission of the International Union of Crystallography [76]. (C) (i) Cartoon of the Fbxo7/PI31 (FP) interacting domain of Fbxo7, (ii) with a model of three Fbxo7 FP domains binding via  $\alpha/\beta$  interfaces (PDB ID: 4L9C and 4L9H). Reprinted with permission of Oxford University Press [59]. (D) Cartoon of the F-box domain of Skp2 (purple) showing three  $\alpha$ -helices, overlaying Skp1 (blue), and in complex with Cul1 (green) and Rbx1 (red) (PDB ID: 1LDK). The  $\alpha$ -helices of one cullin-repeat motif are labelled A-E, and the zinc molecules in Rbx1, which binds the C-terminal globular  $\alpha/\beta$  domain (CTD) of Cul1, are in yellow. Reprinted with permission of Nature Publishing Group [23].

Figure 2. Functions of Fbxo7. Binding of proteins to Fbxo7 can result in various outcomes. 1) Fbxo7 can directly bind to PINK1 and Parkin at the mitochondrial membrane. A loss of mitochondrial membrane potential ( $\downarrow\Delta\psi$ ) results in accumulation of PINK1 at the mitochondria, at which point Fbxo7 aids in the recruitment of Parkin and the induction of mitophagy. The pathogenic mutation T22M inhibits Fbxo7 binding to Parkin, and the R498X mutation inhibits Fbxo7/Parkin accumulation at the mitochondria. 2) Fbxo7 affects the cell cycle through its ability to stabilise active Cdk6/Cyclin D complexes or p27 levels, resulting in oncogenic and tumour suppressive properties respectively. 3) Fbxo7 binds directly to PI31 which can have activating or inhibitory effects on the proteasome. A loss of proteasome regulation is believed to be an important factor in the pathogenesis of PD, resulting in cellular stress and protein aggregation; Fbxo7 involvement in this process is not known. 4) Fbxo7 functions as the substrate specifying subunit of SCF-type E3 ubiquitin ligases, involved in the transfer of ubiquitin from E2 enzymes to substrates. Fbxo7 can mediate the poly-ubiquitination of HURP via K48 linkages, leading to its proteasomal degradation, and also of the NF- $\kappa$ B pathway components cIAP, TRAF2 and NRAGE, potentially via K63 linked ubiquitination. The functional outcome of these modifications changes NF- $\kappa$ B signalling. The R498X mutation may affect substrate recruitment, and the R398G mutation reduces Skp1 binding.

**A****B****C (i)****(ii)****D**