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REVIEW

Cross-functional E3 ligases Parkin and C-terminus Hsp70-interacting protein in neurodegenerative disorders

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Abstract

The study of neurodegenerative disorders has had a major impact on our understanding of more fundamental mechanisms underlying neurobiology. Breakthroughs in the genetics of Alzheimer's (AD) and Parkinson's diseases (PD) has resulted in new knowledge in the areas of axonal transport, energy metabolism, protein trafficking/clearance and synaptic physiology. The major neurodegenerative diseases have in common a regional or network pathology associated with abnormal protein accumulation(s) and various degrees of motor or cognitive decline. In AD, β-amyloids are deposited in extracellular diffuse and compacted plaques as well as intracellularly. There is a major contribution to the disease by the co-existence of an intraneuronal tauopathy. Additionally, PD-like Lewy Bodies (LBs) bearing aggregated α-synuclein is present in 40–60% of all AD cases, especially involving amygdala. Amyloid deposits can be degraded or cleared by several mechanisms, including immune-mediated and transcytosis across the blood–brain barrier. Another avenue for disposal involves the lysosome pathway via autophagy. Enzymatic pathways include insulin degradative enzyme and neprilysin. Finally, the co-operative actions of C-terminus Hsp70 interacting protein (CHIP) and Parkin, components of a multiprotein E3 ubiquitin ligase complex, may be a portal to proteasome-mediated degradation. Mutations in the Parkin gene are the most common genetic link to autosomal recessive Parkinson's disease. Parkin catalyzes the post-translational modification of proteins with polyubiquitin, targeting them to the 26S proteasome. Parkin reduces intracellular $A\beta_{1-42}$ peptide levels, counteracts its effects on cell death, and reverses its effect to inhibit the proteasome. Additionally, Parkin has intrinsic cytoprotective activity to promote proteasome function and defend against oxidative stress to mitochondria. Parkin and CHIP are also active in amyloid clearance and cytoprotection *in vivo*. Parkin has cross-functionality in additional neurodegenerative diseases, for instance, to eliminate polyglutamine-expanded proteins, reducing their aggregation and toxicity and reinstate proteasome function. The dual actions of CHIP (molecular co-chaperone and E3 ligase) and Parkin (as E3-ubiquitin ligase

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Abbreviations used: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; ARJPD, autosomal recessive onset juvenile Parkinson's disease; CDC rel-1, cell division control-related protein; CHIP, C-terminus Hsp70-interacting protein; DLBs, dementia with Lewy bodies; ER, endoplasmic reticulum; HECT, homologous to E6 AP C-terminus; HSPs, heat-shock proteins; LBs, Lewy bodies; Nedd4, neural precursor cell expressed developmentally down-regulated; NFTs, neurofibrillary tangles; Pael-R, Parkin-associated endothelin like receptor; PD, Parkinson's disease; PHFs, paired helical fragments; PINK 1, PTEN-induced putative kinase; p-Tau, phosphorylated tau; RING, really interesting new genes; SOD 1, superoxide dismutase 1; TDP-43, TAR DNA-binding protein; UbcH, ubiquitin-conjugating human enzyme; UPR, unfolded protein response; UPS, ubiquitin proteasome system.

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and anti-oxidant) may also play a role in suppressing inflammatory reactions in animal models of neurodegeneration. In this review, we focus on the significance of CHIP and Parkin as inducers of amyloid clearance, as cytoprotectants and in the suppression of reactive inflammation. A case is made for more effort to explore whether neurodegeneration associated with proteinopathies can be arrested at early stages by promoting their mutual action.
Keywords: CHIP, Neurodegeneration, Parkin, Proteinopathy, Ubiquitin E3 Ligase.
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Protein misfolding, aggregation and gain of toxic function in the brain are strongly allied with neuronal dysfunction. In addition, lost function of normal bystander proteins contributes to neurodegeneration (Olzscha *et al.* 2011). All neurodegenerative disorders are associated with intracellular aggregates of abnormal proteins. For instance, cytoplasmic aggregates of α-synuclein and TAR DNA-binding protein (TDP-43) are found in PD and amyotrophic lateral sclerosis (ALS) (Orr and Zoghbi 2000). Alzheimer's disease (AD) is an exception with prominent amyloid laden plaque depositions in the extracellular compartment. The ubiquitin proteosome system (UPS) has been implicated in each of the major neurodegenerative diseases. Although neurodegenerative disorders individually affect different modules of the brain, the deposition of ubiquitin-associated proteins common to all implies an altered capacity of the neuron to degrade them. The UPS is the major pathway that targets impaired and misfolded proteins for destruction and recycling (Chung *et al.* 2001; Pickart and Cohen 2004; Ciechanover 2005).

The UPS thereby guards cells against the harmful effects of protein accumulation (Furukawa *et al.* 2002; Ciechanover and Brundin 2003). The major proteolytic activity in the UPS is the proteosome. The 26S proteasome is an ATP-dependent complex which is made up of a distinct 20s core and 19s regulatory particles (Goldberg *et al.* 2003). The 26S proteasome has numerous peptidase functions, including trypsin-like, chymotrypsin-like, and peptidylglutamyl-peptide hydrolyzing-like activities (Hershko and Ciechanover 1998). The deficiency to eliminate ubiquitin-conjugated proteins is initially specific to the biochemistry of the target protein in combination with properties unique to that susceptible cell group. Later, the defect may extend to include more generally other essential proteins or even spread throughout a neuronal network. Impairment of the UPS is sufficient to initiate neurodegeneration (Hegde and Upadhya 2011) and early on impairs synaptic activity (Riederer *et al.* 2011).

Ubiquitination is the targeting process for the elimination of unfolded or misfolded protein via the UPS. It occurs through a series of enzymatic reactions involving Ub-activating enzymes (E1), Ub-conjugating enzymes (E2) and Ub-protein ligases (E3) (Hershko *et al.* 2000; McNaught *et al.* 2001). E3 ligases catalyze the final addition of ubiquitin molecules to lysine residues of damaged target proteins. This provides the signal for its removal and degradation by the 26S proteosome (Ciechanover 1998). In addition, E4 is a new ubiquitination enzyme (yeast, Ufd2) that is responsible for multiubiquitin chain assembly (Koegl *et al.* 1999). The U-box present in the conserved motif of Ufd2, is structurally similar to the really interesting new genes (RING) finger motif (Hatakeyama *et al.* 2001; Jiang *et al.* 2001; Murata *et al.* 2001, 2009). Numerous RING finger-containing proteins are assigned to have ubiquitin-protein ligase (E3) activity (Jackson *et al.* 2000; Joazeiro and Weissman 2000).

A well characterized ubiquitin E3 ligase Parkin, is 53 kDa cytosolic protein with known substrates that are targeted for UPS degradation (Shimura *et al.* 2000). Parkin can modify proteins either by attaching polyubiquitin chains (at K48 or K63) or through single or multiple mono-ubiquitinations to achieve alternate biological outcomes (Moore 2006). It is comprised of up to 465 amino acid residues, a C-terminal RING-IBR-RING motif and an N-terminal ubiquitin-like (Ubl) domain (Fig. 1). The C-terminal of Parkin holds two RING domains, common to several E3 ligases, associated with an 'in-between'-RING (Morett and Bork 1999; Ardley *et al.* 2001). The RING domains are cysteine-rich zinc fingers responsible for the recognition of substrate and transfer of monoubiquitin from their attachment with E2 enzymes to substrate while the Ubl domain (residues 1–75) binds to the Rpn10 subunit of the 26S proteasome (Sakata *et al.* 2003). Parkin may act as a neuro-protective agent by contributing to the clearance of α-synuclein and β-amyloid, thus attenuating their toxicity.

Numerous studies have indicated that molecular chaperones known as heat-shock proteins (HSPs) and co-chaperones, for example, C-terminus Hsp70-interacting protein (CHIP), also play a vital role in maintaining the protein homeostasis in a swarming cytoplasmic milieu. The CHIP is a 34.5-kDa and ubiquitously expressed cytosolic protein (Ballinger *et al.* 1999) having dual function as both a molecular co-chaperone and ubiquitin E3 ligase. The N-terminal domain of CHIP consists of three tetratricopeptide domain repeats that are involved in the protein–protein interaction with various molecular chaperones (Hsp70/90) and co-chaperones including Hip, Hop, Cyclophilin 40, FKBP52 and Bag1 phosphatase 5 (Ballinger *et al.*1999; Connell *et al.* 2001; Kampinga *et al.* 2003; Dai *et al.* 2005; Hwang *et al.* 2005). The C-terminal part consists of a single RING finger or U-box domain responsible for catalyzing the transfer of ubiquitin to misfolded or aggregated client proteins destined for elimination (Aravind and Koonin 2000; Jiang *et al.* 2001). Owing to this bifunctionality, CHIP behaves as a molecular triage,

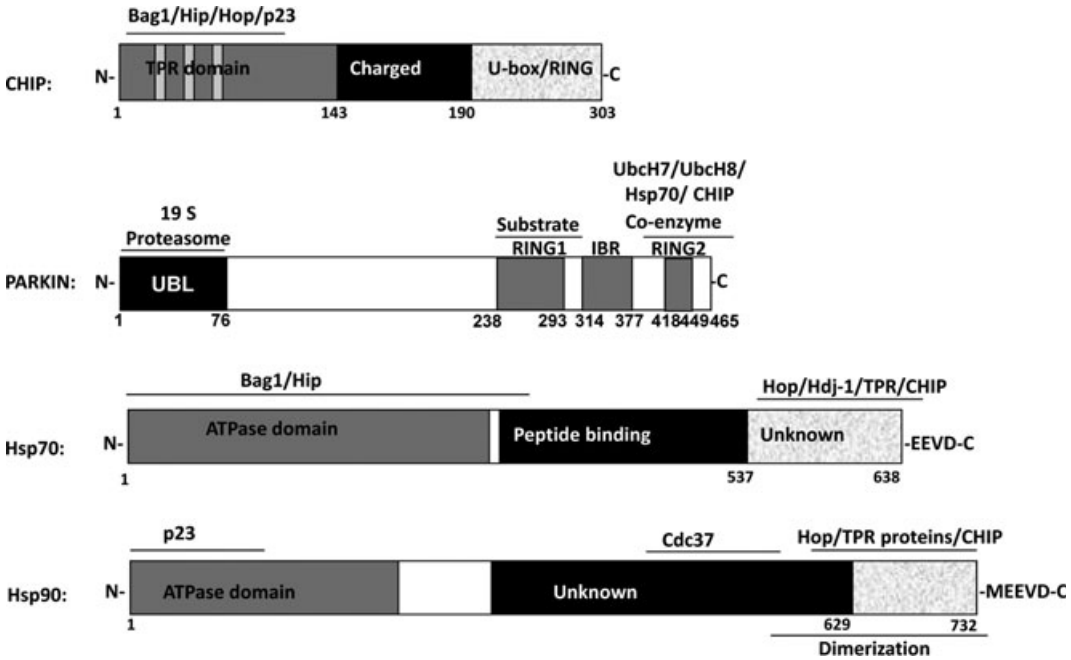

Fig. 1 The domain structure of E3 ligase CHIP, Parkin and heat-shock proteins Hsp70 and Hsp90. CHIP consists of three TPR domains that are responsible for protein-protein interaction while its C-terminal part is composed of a U-box or Really interesting new genes (RING) finger domain that facilitates client protein destruction. Parkin, another E3 ligase consists of an Ubl domain at the N-terminus followed by two RING finger regions separated by an 'in-between'-RING (IBR) domain in its C-terminus. The RING 1 domain binds substrate whereas RING 2 interacts with co-enzyme components (e.g. E2) and chaperone proteins. Hsp70 and Hsp90 contain ATPase domains in its N-terminal region followed by a common consensus sequence EEVD C-terminus that is responsible for various co-chaperonic activities.

orchestrating protein fate through attempts to refold versus degrade misfolded proteins (McClellan and Frydman 2001; McDonough and Patterson 2003).

CHIP, in collaboration with Hsp70 and Hsp90, polyubiquitinates a number of misfolded and aggregated protein targets thereby enhancing cell survival. These include gene products with expanded polyglutamine tracts responsible for Huntington's disease and hereditary ataxias (Sakahira *et al.* 2002; Jana *et al.* 2005; Miller *et al.* 2005; Morishima *et al.* 2008). CHIP polyubiquitinate phosphorylated 4-repeat-Tau, alleviates Tau aggregation and reverses neuronal toxicity (Hatakeyama *et al.* 2004; Petrucelli *et al.* 2004; Shimura *et al.* 2004; Sahara *et al.* 2005). The immature form of cystic fibrosis transmembrane conductance regulator is also targeted by CHIP for degradation (Meacham *et al.* 2001). Proteasomal degradation of the tumor suppressor p53 is regulated by CHIP as well as by another ligase Mdm2 (Esser *et al.* 2005). Finally, CHIP orchestrates the degradation of α-synuclein by triaging between the proteasomal and lysosomal pathways (Shin *et al.* 2005).

Ubiquitin E3 ligases in neurodegenerative disorders

Alzheimer's disease

AD is clinically manifested by progressive loss of memory and other cognitive skills, resulting in severe dementia. The cerebral decline is accompanied by the progressive accumulation of insoluble fibrous material in the brain in the form of senile plaques and Neurofibrillary tangles (NFTs). Amyloid β peptides are self-aggregating molecules and form the major component of amyloid fibrils found in diffuse, cored and senile (neuritic) plaques and in blood vessel walls. The two protein lesions have synergistic effects in AD (Ittner and Götz 2011). Aβ induced neurotoxicity is associated with endoplasmic reticulum (ER) stress and mitochondrial dysfunction (Kadowaki *et al.* 2005; Reddy 2007).

Aβ is generated by endoproteolysis of amyloid precursor protein (APP), accomplished by the sequential cleavage of APP by the secretase group of enzyme complexes. In the so-called non-amyloidogenic pathway, APP is processed by α-secretase. This releases a large soluble N-terminal APP ectodomain fragment (sAPPα) and cleaves within the Aβ sequence, thus preventing formation of Aβ by γ-secretase processing. In the amyloidogenic path, the proteolytic cleavage of APP by β-secretase (also known as β site APP cleaving enzyme 1) releases sAPPβ, and subsequently γ-secretase (presenilin complex) acts on the remaining membrane bound portion of APP (C99) to yield an intact Aβ peptide. Amyloid is generated in early endosomes and/or ER-golgi intermediate compartments and then released into the extracellular spaces (Selkoe 1999; Haass and Selkoe 2007; Querfurth and LaFerla 2010). Endocytic trafficking of membrane bound APP is

another major source (Koo *et al.* 1996). Aβ peptides of varying lengths are produced by a sequential intramembranous processing mechanism inherent to the γ-secretase complex (Takami *et al.* 2009). The longer $A\beta_{1\text{-}42}$ peptide is more prone to fibril formation and is also the isoform predominantly found in cerebral plaques. The α-secretase activity is mostly localized to the cellular surface, whereas β- and γ-secretase activities are primarily found in endosomal compartments. Accordingly, altered subcellular trafficking of APP directly influences the degree to which Aβ is generated (Hermey 2011). $A\beta_{42}$ induces free radical formation, oxidative stress and the ER stress response (Behl *et al.* 1994; Mattson and Goodman 1995; Ghribi *et al.* 2003; Esposito *et al.* 2004; Hoshino *et al.* 2007; Chafekar *et al.* 2008).

The association of Aβ, ubiquitin and α-synuclein in AD plaque deposits and co-existence of diffuse amyloid plaques with ubiquitin-positive LBs in dementia with Lewy bodies (DLBs) (Harrington *et al.* 1994; Gomperts *et al.* 2008; Lashley *et al.* 2008; Burack *et al.* 2010) have long suggested the possibility that the proteasome and ubiquitin ligases may be relevant to the biology of both AD and PD. The proteasome apparatus is closely approximated or directly in contact with the ER and receives exported proteins that were incorrectly folded. Investigations into the role of proteasome metabolism in amyloid clearance (Gregori *et al.* 1995; Favit *et al.* 2000) led to the discovery of a role for ubiquitin ligases in the reduction of extra and intracellular Aβ. Amyloid plaque burden was lessened by Parkin in an *in vivo* study (Burns *et al.* 2009). Surprisingly however, absence of Parkin resulted in improvement in plaque and phospho-Tau pathology in the AD-like APP (swed) transgenic mouse (Perucho *et al.* 2010). The authors attributed this result to the induction of CHIP, another E3 ligase, and chaperones to compensate for the Parkin deficiency. The suppression of Parkin decreased amyloid plaques in cortex and hippocampus, as well as soluble and total Aβ levels by ELISA in APP swed mice (Perucho *et al.* 2010). Interestingly, levels of aggregated Aβ were either unchanged or slightly increased in Parkin knockdown mice (Perucho *et al.* 2010). *In vitro* and cell culture work has recently shown that the degradation of *intracellular* Aβ is facilitated by Parkin through ubiquitination and proteasomal degradation (Burns *et al.* 2009; Rosen *et al.* 2010). CHIP also quickens the clearance of cellular Aβ in a manner consistent with its known neuroprotective properties. It has switching roles to stabilize normal APP levels or to act as an intermediate in the ubiquitination of an unwanted pool of APP or to lessen levels of cytotoxic β-amyloid. The practical significance of CHIP and client chaperones is to maintain steady state levels of APP during oxidative stress and attenuate Aβ toxicity (Kumar *et al.* 2007) (Fig. 2).

NFTs which contain hyperphosphorylated Tau protein in the form of paired helical fragments (PHFs) (Ihara 2006) are another target of CHIP and/or Parkin. The 'Tau axis hypothesis' comprises two parts; first, there is the Tau dependent mediation of amyloid β toxicity and second, exposure of neurons to β-amyloid enhances hyperphosphorylation of Tau. According to this hypothesis, interaction of β-amyloid and Tau contributes to failure of axonal transport that eventually leads to mitochondrial dysfunction (Ittner and Götz 2011). It has first been observed that the presence of Aβ

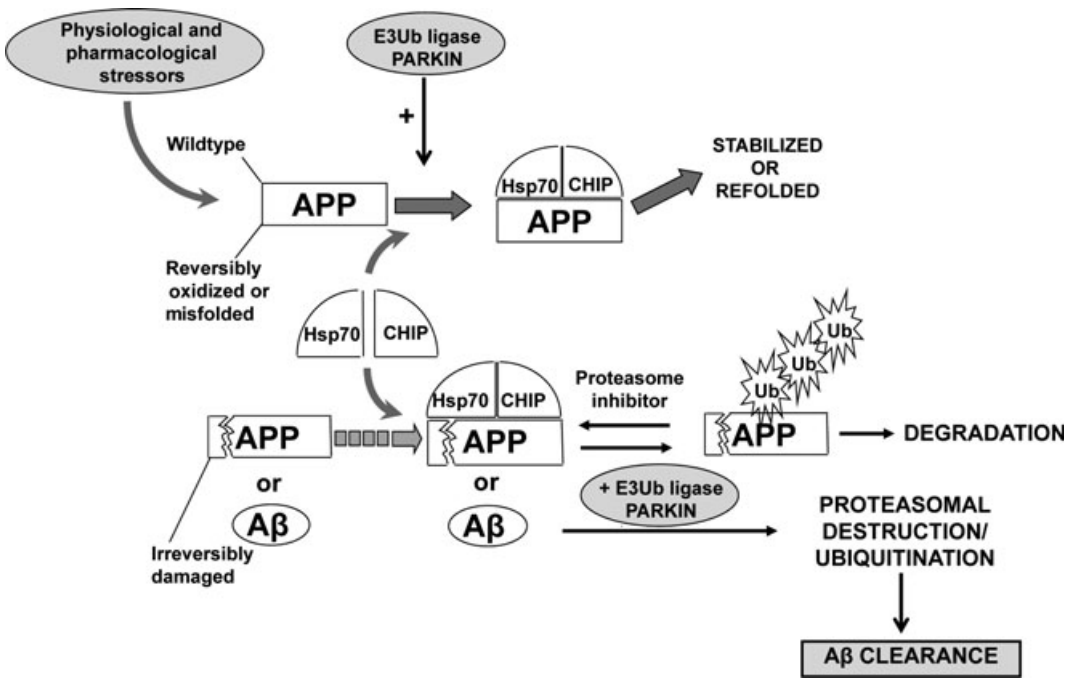

Fig. 2 Model for Parkin and CHIP-dependent protection against Aβ-induced toxicity (Kumar *et al.* 2007 modified).

induces the action of various protein kinases to abnormally hyperphosphorylate Tau (Götz *et al.* 2001). Then, it came to the Mucke *et al.* group to show that Tau was necessary for the expression of amyloid toxicity (Roberson *et al.* 2007; Vossel *et al.* 2010). One linking mechanism comes from the observation that accumulation of Aβ decreased the expression of CHIP that ultimately led to increases in abnormal Tau levels. Conversely, restoration of normal CHIP levels mitigated Aβ induced toxicity by reducing the Tau dependency (Oddo *et al.* 2008). Parkin also reduces Tau phosphorylation in a background of combined Aβ and α-synuclein protein stressors (Burns *et al.* 2009; Moussa 2009).

E3s and tauopathy

Tau is a microtubule associated protein that plays a crucial role in stabilizing the microtubules and thus the cytoskeletal structure of the cell. This cytosolic protein is under the protection of molecular chaperones Hsp 70 and 90 that maintain proper functioning and prevent misfolding (Salminen *et al.* 2011). Once these HSPs are inundated with excess Tau, the formation of Tau oligomers follows (Sahara *et al.* 2007). Loss of normal and/or gain of toxic Tau functions result in the 'Tauopathy' group of neurodegenerative disorders. Tauopathy is commonly seen in AD, where the Tau protein is hyperphosphorylated and forms PHFs. NFTs are cytoplasmic lesions first appearing within the neurons of the entorhinal cortex, hippocampus, amygdala, and several subcortical nuclei. The action of various Tau kinases and loss of phosphatases promote the formation of PHFs. An inflammatory environment probably abets the process (Ballatore *et al.* 2007). The kinases that promote Tau hyperphosphorylation include glycogen synthase kinase-3β (GSK-3β), cdk5, and Microtubule Affinity Regulatory Kinases.

Hyperphosphorylated Tau dissociates from microtubules. Impaired activities of molecular chaperones (HSPs) and co-chaperones (CHIP, Parkin, Hip and Hop) contribute to the formation of NFTs. These molecular chaperons work together with the UPS in order to degrade NFTs (Muchowski and Wacker 2005; Goryunov and Liem 2007). Thus, increases in the levels of Hsp 90 and Hsp 70 reduce NFT load (Dou *et al.* 2003). Although HSPs are involved in refolding hyperphosphorylated Tau, the failure to do so calls into action CHIP which associates with the same HSPs to promote ubiquitination and degradation (Dickey *et al.* 2007a,b; Goryunov and Liem 2007). It has been observed that CHIP interacts differently with Hsp 70 and Hsp 90, but through the same tetratricopeptide domain repeat domain. This switch could be related to CHIP's triage ability. The CHIP–Hsp70 complex may correspond to its general protein refolding activity, whereas the CHIP–Hsp 90 complex seems to regulate the degradation of signal transduction protein substrates (Connell *et al.* 2001; Young *et al.* 2001; Dickey *et al.* 2007a,b). The inhibition of Hsp90 becomes important for the clearance of aggregated Tau as observed in mouse models of Tauopathies (Luo *et al.* 2007). During stress, elevated levels of cellular kinase Akt disrupt CHIP–Hsp 90 complexes, in turn activating glycogen synthase kinase-3β (GSK-3β) and leading to the hyperphosphorylation of Tau (Dickey *et al.* 2008). Kosik and Shimura (2005) reported that phosphorylated sites on Tau act as a signal for ubiquitination. Hsp 70 binds only to hyperphosphorylated-Tau and not to the non-phosphorylated Tau. This assists the formation of a CHIP–Hsp70–p-Tau complex, allowing for the ubiquitination and successive degradation of p-Tau. The cell is thereby rescued from toxicity and death (Shimura *et al.* 2004; Kosik and Shimura 2005). There is an inverse relation between CHIP and p-Tau levels that was observed in CHIP null mice, where there was recorded increased level of p-Tau and declines in Hsp 70 levels (Sahara *et al.* 2005; Dickey *et al.* 2006). Over-expression of CHIP resulted in degradation of hyperphosphorylated as well as normal Tau, without any net disturbance of spatial memory in mice models (Zhang *et al.* 2008a,b).

Petrucelli *et al.* (2004) reported that Parkin is also an important E3 Ubiquitin ligase that acts on p-Tau. This was somewhat contradicted by Shimura *et al.* (2004) stating that Tau was absent in co-immunoprecipitates using anti-Parkin antibodies. Moreover, ubiquitination of Tau under *in vitro* conditions is regulated by CHIP/Hsp 70 complex and not by Parkin (Shimura *et al.* 2004). However, double transgenic mice over-expressing mutated Tau and lacking Parkin ($PK^{-/-}$/Tau^{VLW}) had lower levels of CHIP–Hsp 70 complex and increased oxidative stress, triggering Tau accumulation and aggregation. The aggrevated deposition of Tau occurred in the hippocampus and substantia nigra in a perinucleus location (Menéndez *et al.* 2006; Guerrero *et al.* 2008; Rodríguez-Navarro *et al.* 2008).

As Parkin associates readily with CHIP (Imai *et al.* 2002) and seems to play a protective role in substantia nigra dopamine neurons against a degenerative Tau background (Klein *et al.* 2006), we conclude that Parkin probably does have some key role in Tau metabolism. Parkin, apart from being an E3 Ubiquitin ligase, also assists binding of Tau to the actin cytoskeleton. The absence of Parkin predictably hinders this association and further leads to the impairment of cellular transport necessary for survival (Huynh *et al.* 2000; Yang *et al.* 2005; Menéndez *et al.* 2006).

As alluded to earlier, a number of studies have shown that introduction of exogenous Aβ resulted in increased Tauopathy (Götz *et al.* 2001). Conversely, the transgenic expression of Tau did not have any effect on the levels of Aβ or its pathology (Oddo *et al.* 2007). Parkin is shown to carry an important role in attenuating the toxic effect of β-amyloid in cultures of skeletal muscles and neurons through proteasome elimination (Rosen *et al.* 2006). However, Parkin deficiency or mutations aggrevate amyloid accumulation. Moreover, Parkin levels are reduced in AD brain regions with high loads of β-amyloid (Rosen *et al.* 2010). From this, it is predicted that Parkin stimulation should also attenuate

Aβ-induced Tauopathy and some progress in the latter has been presented (Moussa 2009). In summary, both Parkin and CHIP are potentially important in controlling loss of normal Tau function and gain of toxic function paradigms in AD.

Parkinson's disease

PD, the second most common neurodegenerative disease after AD is characterized by the degeneration of dopaminergic neurons in the substantia nigra, pars compacta and the accumulation of cytoplasmic proteinaceous inclusions known as LBs (Moore *et al.* 2005). The α-synuclein protein is the major component of LBs, lewy neuritis and filamentous inclusions and dominant missense mutation of SYN causes disease in a small percent of familial cases (Goedert 2001). Other genes, Parkin, PTEN-induced putative kinase (PINK)-1 and DJ-1, when mutated lead to Autosomal recessive juvenile oncet Parkinson's disease (ARJPD; Cookson and Bandmann 2010). Parkin protein is present in the LBs of ARJPD, DLB and sporadic forms of PD (Shimura *et al.* 2001; Schlossmacher *et al.* 2002; Klein and Schlossmacher 2007). Parkin action to limit LB formation is dependent on proteasome activity (Ardley *et al.* 2003). Compound heterozygous mutations result in loss of Parkin function and are the most common genetic cause (∼50%) of ARJPD (Kitada *et al.* 1998; Lücking *et al.* 2000). Recently it has been reported that Parkin act as a p53 repressor and independent to E3 ubiquitin ligase function in ARJPD (da Costa *et al.* 2009). Although Parkin dysfunction is clearly implicated in ARJPD, the precise pathogenesis of PD remains obscure and controversial (Lim 2007; Matsuda and Tanaka 2010). Parkin promotes degradation and clearance of multiple substrates, including but not limited to: mutated α-synuclein (A53T) and glycosylated synuclein-22, synphilin, Parkin-associated endothelin-like receptor (Pael-R), cell division control-related protein (CDC rel-1) (Imai *et al.* 2000, 2001), and a polyglutamine-expanded mutant of ataxin-3 (Shin *et al.* 2005). Parkin mutations result in loss of E3 ligase activity and lead to the degeneration of dopaminergic neurons. The effect of such mutations in transgenic flies is rescued by increased expression of glutathione S-transferase suggesting oxidative damage underlies the phenotype (Whitworth *et al.* 2005). Conversely, Parkin knockout mice experiments reveal decreased oxidative phosphorylation and increased oxidative stress (Palacino *et al.* 2004).

Parkin directly modulates 26S proteasome activity (Um *et al.* 2006, 2010). The interrelation between Parkin activity, ubiquitination, the UPS and mitochondrial function is being increasingly explored to address protein aggregation and organelle failure in neurodegenerative diseases. Several recent studies revealed that Parkin actions include facilitated throughput in the aggresome-autophagy-lysosome system and mitophagic removal of defective mitochondria (Chin *et al.* 2010; Khandelwal and Moussa 2010). The latter occurs via a PINK-1-dependent mechanism recognizing the loss of mitochondrial membrane potential (Matsuda and Tanaka 2010). *Drosophila* research showed that Parkin also protects cells from agents initiating the mitochondrial death pathway (Greene *et al.* 2003, 2005).

Therefore, Parkin expression and gain-of-function may have therapeutic application across proteasome, autophagy and mitochondrial systems.

In sporadic PD, there is evidence as well for Parkin dysfunction. Cysteine residues in the RING domains of Parkin are susceptible to nitrosative and oxidative modifications that change protein function. The modification of E3 ligase activity by nitric oxide links an environmental stressor to a molecular abnormality similar to hereditary forms of PD (Chung *et al.* 2004; Yao *et al.* 2004). Parkin is dependent on E2 ubiquitin-conjugating human enzyme 8 (UbcH8) through interaction with its C-terminal R2 RING-finger domain. UbcH8 expression is normally high in the central nervous system (Kimura *et al.* 1997), whereas UbcH7 levels are in the low detectable range (Katsanis and Fisher 1998). This specificity may have some role in the interesting observation that Parkin is able to self-ubiquitinate in an E2-dependent manner, thus regulating its own degradation. Familial-linked and RING-finger-containing cysteine mutations, especially within R2 domain, interrupt this ability of Parkin to target itself for elimination. Parkin controls the turnover of proteins important to synaptic function, such as CDCrel-1, and the same mutations negatively affect this function (Zhang *et al.* 2000). Despite the identification of ubiquitin conjugates in the post-synaptic density and synaptic terminals in rodent brain (Chapman *et al.* 1992, 1994), it is unclear whether E3 ligase dysfunction in ARJPD or even in sporadic PD and CDCrel-1 turnover are essential to disease expression. Whether modifications in the turnover of CDCrel-1 actually contribute to the degeneration of dopamine neurons is also unsettled.

An important study examining familial PD mechanisms reported that Hsp70 rapidly binds to unfolded Pael-R, hindering access to Parkin's E3 activity. The bifunctional co-chaperone CHIP aids the dissociation of Hsp70 from this complex with Pael-R and Parkin, fostering Parkin-mediated Pael-R ubiquitination (Fig. 3). The authors liken CHIP to a mammalian E4-like molecule that directs Parkin E3 activity (Imai *et al.* 2002). ARJPD mutation of Parkin results in Pael-R accumulation in human brain (Imai *et al.* 2001). Nevertheless, the causal link between Pael-R toxicity and PD pathology remains uncertain. It is also proposed that Parkin may lead to proteasomal clearance of α-synuclein (Petrucelli *et al.* 2002), but Parkin hypofunction in sporadic PD is not proven and the nature of the abnormality targeting non-mutant Synuclein is speculative. CHIP on the other hand certainly plays a key role in recognizing and modulating degradation of toxic, oligomeric forms of α-synuclein (Shin *et al.* 2005; Tetzlaff *et al.* 2008). AD and PD aside, both CHIP and Parkin have promising effects on other proteinopathies.

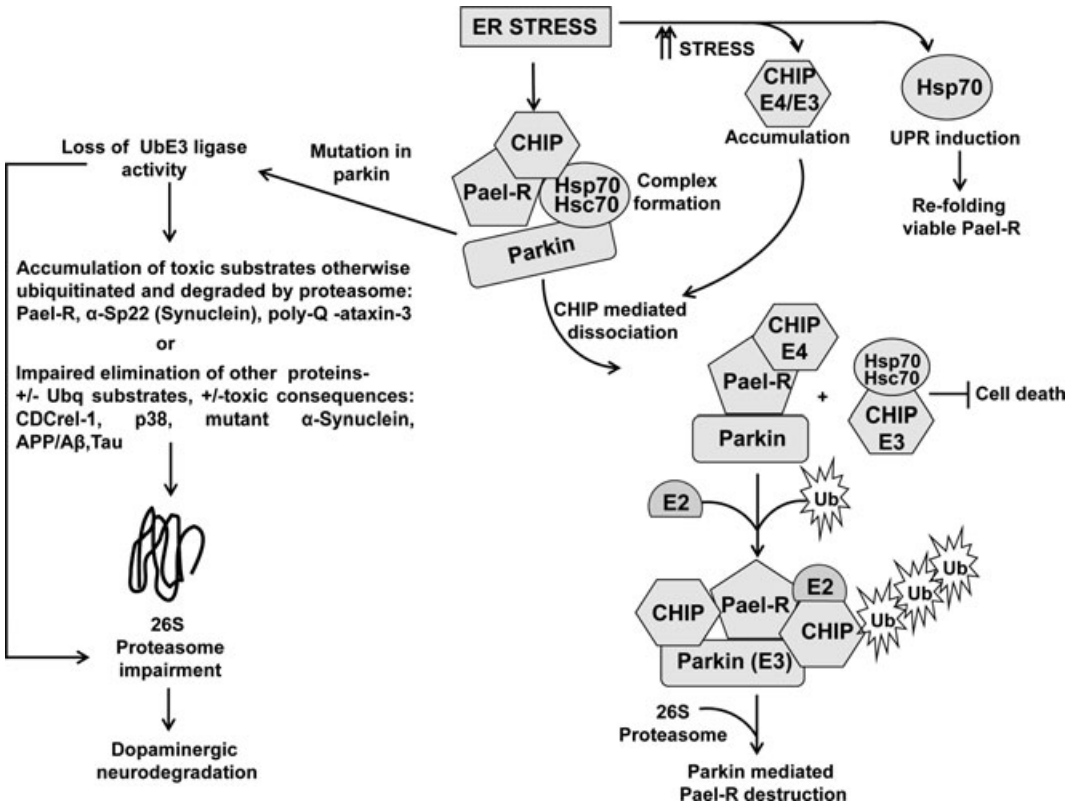

Fig. 3 Ubiquitin E3 ligase activities of CHIP and Parkin are called to action in PD. ER stress leads to the accumulation of CHIP in the cytoplasmic milieu. A complex is formed involving the Pael-R receptor, Hsp70, CHIP (E3 and E4 like activity) and Parkin protein. This complex is further dissociated by increased level of CHIP. A conjugating enzyme E2 along with 26S proteasome enhances the clearance of mutated or damaged Pael-R through the ubiquitination process while CHIP–Hsp70 complex is released into the cytoplasm. Ubiquitin E3 ligase activity is lost once the Parkin gene is mutated. This results in the accumulation of several defective receptor proteins that rely on UPS for degradation.

Amyotrophic lateral sclerosis

ALS, the third most common neurodegeneration, is the most lethal and characterized by progressive paresis of limb and bulbar muscles. It is a disease largely restricted to motor neurons of the cerebral cortex, brainstem and spinal cord but lies on a spectrum of additional pathlogy involving the frontotemporal lobes (Ferrari *et al.* 2011; Kiernan *et al.* 2011). The signature lesion in both sporadic and familial forms is the cytoplasmic ubiquitinated inclusion immunoreactive for the TDP-43 (Janssens *et al.* 2011).

Mutation of the copper-zinc superoxide dismutase-1 (SOD1) gene is the most common genetic form, causal in 20% of autosomal dominant familial ALS as well as in some sporadic cases (Rosen 1993). A significant percent of additional familial ALS cases are caused by mutations in TDP-43 or fused-in-sarcome/translocated in liposarcoma (Lagier-Tourenne *et al.* 2010).

Most information we have regarding ALS gene product degradation pathways is on mutant SOD1, which is still the best characterized transgenic model for the disease (Peviani *et al.* 2010). SOD1 is an antioxidant enzyme, but there is much evidence supporting a 'gain of toxic function hypothesis' rather than a 'loss of function' when SOD-1 is mutated (Cleveland and Rothstein 2001; Julien 2001). Thus, assisting SOD-1 degradation should prove neuroprotective. Metal (Cu/Zn^{2+})-free, monomeric SOD-1 (wt or mutant protein) is degradable in a proteasome-dependent manner but not requiring ubiquitination (Di Noto *et al.* 2005). Next, it was reported that Hsp70 or heat-shock cognate Hsc70, and CHIP are involved in proteasomal degradation of mutant SOD1 but not wt SOD (Urushitani *et al.* 2004). Furthermore, only the mutated form of SOD-1 was shown to interact with Hsp70, CHIP or in complex involving all three. Apparently here as well, it is the Hsp70 that gets polyubiquitinated by CHIP, not SOD1 directly. The result however, is the delivery of misfolded SOD-1 to the 26S-S5a particle proteasome for degradation. Similar indirect ubiquitination and reduction in SOD toxicity attributed to CHIP was found by another group (Choi *et al.* 2004). An added aspect is that mutant SOD-1 is damaging to the proteasome itself. There was observed a decrease in expression of proteasome subunits and impaired function of the ubiquitin-proteasome pathway in spinal motor neurons of transgenic mice harboring the SOD gene mutation G93A (Cheroni *et al.* 2009). Other E3 ligases accelerate mutant SOD-1 degradation. Dorfin ubiquitinates SOD-1 proteins and in the same transgenic model reduces

SOD-1 levels in and degeneration of spinal motor neurons (Sone *et al.* 2010). At the subcellular level, Dorfin reduces mitochondrial SOD1 levels and toxicity (Takeuchi *et al.* 2004). Dorfin immunoreactivity decorates inclusions in familial ALS and sporadic ALS (Niwa *et al.* 2002). Gp78 is an ER associated E3 ligase which also promotes SOD (as well as Ataxin-3) degradation as part of the endoplasmic reticulum associated domain response path (Ying *et al.* 2009). Accordingly it is induced in cells harboring mutated SOD-1.

Cytoplasmic TDP-43 aggregates are polyubiquitinated in ALS. Although proteasome inhibition may increase their load, the deposits are associated to greater degree with the autophagosome system and autophagy inhibitors are more active. Thus, for TDP-43 at least, autophagy-mediated clearance may be more important (Urushitani *et al.* 2010). Nevertheless, dysfunction of TDP-43 may cause impairment of the ubiquitin-proteasome pathway, providing a link to neurodegeneration (Wang *et al.* 2008a,b; Buratti and Baralle 2009). There is little information on ubiquitin ligase action on TDP-43. In summary, the function of these protein aggregates in the ALS spectrum is still ambiguous and their precise relation to the UPS and autophagy-lysosomal systems for degradation remains to be clarified.

Huntington's disease

Huntington's disease is a polyglutamine (polyQ) expansion disorder in which accumulation of mutated huntingtin protein (htt) in neurons results in both nuclear and cytoplasmic toxicity through numerous mechanisms (Li and Li 2006; Imarisio *et al.* 2008). Nuclear htt aggregates are decorated with ubiquitin and depressed proteasome activity is observed in some (Wang *et al.* 2008a,b) but not all models (Li and Li 2011). Degeneration of striatal neurons results in progressive motor, cognitive and psychological symptoms culminating in early death (Novak and Tabrizi 2010).

A mutant expansion of CAG repeats encoding abnormal long stretches of glutamine (polyQ) results in htt misfolding and aggregation. Other autosomal dominant polyQ diseases with similar disease mechanisms include spinocerebellar ataxia type 3 (SCA-3 or Machado-Joseph disease [MJD] from expanded ataxin) and, spinal and bulbar muscular atrophy. In addition to UPS dysfunction (Ciechanover and Brundin 2003), other hypotheses for neuronal degeneration in polyQ diseases include: misfolding of the mutant protein leading to changes (gain or loss) in its function; unfavorable protein interactions seeded by the mutant protein; formation of toxic oligomer complexes; dysregulation of transcription; mitochondrial dysfunction resulting in oxidative stress; and impairment in the maintenance of other protein homeostatic mechanisms including autophagy and RNA toxicity (Gatchel and Zoghbi 2005; Bennett *et al.* 2007; Pandey *et al.* 2007; Todi and Paulson 2007; Todi *et al.* 2007; Li *et al.* 2008; Zuccato *et al.* 2010).

Complementary cellular and animal model experiments support the notion that CHIP is a significant component of the neuronal quality control machinery. In its triage role, CHIP maintains protein homeostasis within a number of misfolded protein pathways. For instance CHIP has beneficial effect to reduce polyQ aggregation and toxicity (Miller *et al.* 2005; Jana *et al.* 2005; Al-Ramahi *et al.* 2006; Choi *et al.* 2007; Branco *et al.* 2008). CHIP may also differentially control polyQ protein metabolism depending on protein context (Bulone *et al.* 2006; Dickey *et al.* 2007a,b; Branco *et al.* 2008; Robertson and Bottomley 2010). In other neurodegenerative diseases, CHIP has been shown to monitor disease proteins including α-synuclein, Parkin and Tau (Imai *et al.* 2002; Shin *et al.* 2005; Dickey *et al.* 2006, 2007a,b, 2008; Tetzlaff *et al.* 2008). Mutant PolyQ, similar with α-synuclein and Aβ, tends to misfold and oligomerize. In a toxic oligomer model of polyQ disease pathogenesis, CHIP had a major role to diminish levels of these complexes (Williams *et al.* 2009). Parkin expression also promotes the degradation of polyglutamine-expanded protein aggregates via ubiquitination and restores proteosome function, thus averting endoplasmic reticulum- stress associated cell death (Tsai *et al.* 2003).

Crossing disease lines: Parkin and amyloid β

Parkin has pan-protective properties against a broad range of toxic insults even when these are not directly connected to the UPS or PD (Hyun *et al.* 2002; Darios *et al.* 2003; Staropoli *et al.* 2003; Manfredsson *et al.* 2007). Similarly, neither α-synuclein pathology nor a relative loss of Parkin function is confined to PD. For instance, although LBs laden with α-synuclein are the defining pathological lesion in PD and DLB, LBs are observed in ~60% of sporadic or familial AD brains and 13% of cognitively normal aged individuals, as reported in both community and pathologic series. The amygdala is especially prone but LBs are also found in entorhinal, neocortical, and brainstem areas (Hamilton 2000; Kotzbauer *et al.* 2001; Tsuang *et al.* 2006; Uchikado *et al.* 2006). Conversely, amyloid plaque number and burden are clearly increased in ~2/3 of PD with dementia patients or DLB cases using the Pittsburgh compound B positron emission tomography (PIB-PET) imaging technique (Gomperts *et al.* 2008; Burack *et al.* 2010). In PD alone, 50% of cases manifest high correlation between cortical Synuclein load and diffuse amyloid plaque burden (Lashley *et al.* 2008). Elevated brain levels of $A\beta_{1-42}$ in familial Lewy body disease (LBD) (Kaneko *et al.* 2007) further illustrates the concept that α-synuclein and Aβ accumulations might be inter-connected. There is even cross-association between α-synuclein burden and NFT load in familial PD (Duda *et al.* 2002).

It is clear that at the mitochondrial level and even in the absence of misfolded protein stress, Parkin expression prevents oxidative injury and mitochondrial dependent apoptosis. Conversely, absence of Parkin worsens the same (Darios *et al.* 2003; Palacino *et al.* 2004). The restoration of

ATP levels, however, is not sufficient to mitigate proteasome dysfunction which is also a consequence of $A\beta_{42}$ buildup. Thus, mitochondrial ATP production is not limiting at a time when the proteasome is under aggregated protein attack. Early reports applied synthetic amyloid to non-neuronal cell lines to show inhibition of substrate degradation by the 20s proteasome (Gregori *et al.* 1995; Lopez Salon *et al.* 2003; Schmitz *et al.* 2004).

Intracellular $A\beta_{42}$ also inhibits proteasomal activity both *in vivo* and *in vitro* (Rosen *et al.* 2010). In theory, however, any misfolded proteinopathy involving oxidative damage can hinder proteasomal activity (Ding and Keller 2001). Not only does Parkin sustain proteasome function under $A\beta_{42}$ stress it also stimulates it under normal conditions. A key process to rapidly ubiquitinate abnormal proteins in the ER is through endoplasmic reticulum associated degradation system involving retro-translocation to the proteasome for proteolysis. In some cases, chaperones accompany and aid in the polyubiquitination of soluble substrates as they shuttle to the cytoplasmic 26S proteasome. Small membrane-bound peptides can even bypass ubiquitination and be directly accompanied by ER-associated E3 ligases or co-chaperones to the 19s proteasome cap. In the case of intracellular $A\beta_{42}$, there is recent evidence from immunoprecipitation experiments and mass spectrometry (tandem MS) of covalently modified lysine residues (Rosen *et al.* 2010) (Fig. 4) as well as from MS using peptide mass fingerprinting and fragment ion analysis (Burns *et al.* 2009; Khandelwal *et al.* 2011), that a fraction thereof is mono ubiquitinated.

Parkin associates with mitochondrial membranes (Darios *et al.* 2003), where an interaction with the PINK-1 gene shields mitochondrial function from oxidative stress damage (Winklhofer and Haass 2010). In the presence of dopaminergic stress, the non-receptor kinase c-Abl phosphorylates Parkin on tyrosine 143 to inhibit both its E3 ligase activity and protective function. Conversely, the c-Abl-family kinase inhibitor STI-571 averts abnormal phosphorylation of Parkin, thereby maintaining its dual functions (Ko *et al.* 2010).

It is not known how these or other post-translational modifications of Parkin, such as from oxidative damage, affect its role in amyloid processing. However, mutation of Parkin (T240R: RING1 domain) renders it ineffective at reducing Aβ load and unable to rescue 20s proteasome activity (Burns *et al.* 2009; Rosen *et al.* 2010). As expected, chemical inhibitors of the proteasome similarly block these functions of wt Parkin. Native Parkin binds soluble monomeric and oligomeric forms of Aβ but not with the insoluble, fibrillar Aβ species nor is it to be found associated with advanced plaques. In fact, Parkin levels appear overall

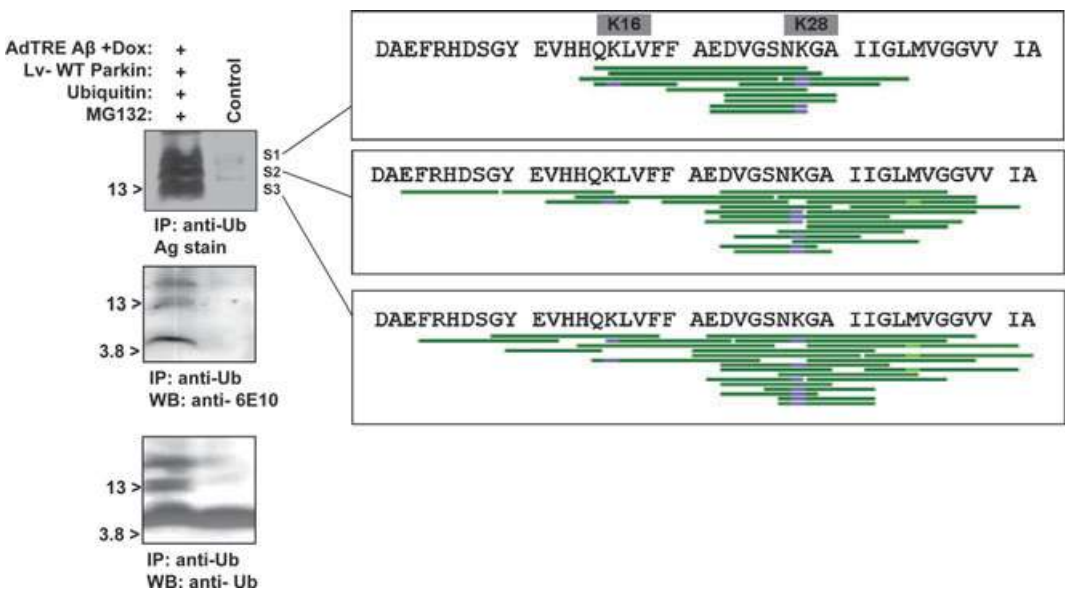

Fig. 4 Monoubiquitin modifications at Aβ lysine residues 28 and 16. SH-SY5Y lysates were fractionated on 4–12% NuPAGE and immunoprecipitated with anti-ubiquitin antibody. Cells were infected with AdTRE Aβ: adenovirus tetracycline response element encoding human β-amyloid $_{1\text{-}42}$ (Tet-on + Doxycycline) and/or Lv-WT Parkin: lentivirus encoding wild type Parkin; co-transfected with Ubiquitin cDNA and treated with proteasome inhibitor MG132. The three silver stained bands (S1, S2, S3; MW 13∼21 kDa) were excised and submitted for LC/Tandem MS analysis (nanobore electrospray and Thermo LTQ ion trap). Ctl: IP from control lysates (AdTREAβ and MG132 only, absent Dox, Parkin, or Ubq) followed by western detection: monoclonal anti-ubiquitin and anti-β-amyloid (6E10). Aβ sequence-containing peptide fragments identified by MS/MS are shown in green; where modified by ubiquitin, in blue. Fragmentation spectra were searched against NCBI database using SEQUEST algorithm. The predicted mass of the ∼4 kDa human β-amyloid modified by one or two 8.6 kDa Ubq molecules is 13–21 kDa (Rosen *et al.* 2010, diagram courtesy of Dr Jon DeGnore, Tufts University School of Medicine).

depressed in AD brain (Rosen *et al.* 2010). What Parkin is available, appears to be tied up with intracellular Aβ suggesting a direct substrate-ligase relationship in AD pathogenesis. AD brain is also deficient in proteasomal activity (Keller *et al.* 2000a,b; López Salon *et al.* 2000). Furthermore, neuritic plaques and NFT structures harbor ubiquitin and its conjugates (Perry *et al.* 1985, 1987; Morishima-Kawashima *et al.* 1993).

These studies are consistent with the rationale to therapeutically boost Parkin and/or proteasome activity in PD and other neurodegenerations (Butcher 2005; Kirik and Björklund 2005; Winklhofer 2007; Ulusoy and Kirik 2008). The endogenous proteasome system to degrade amyloid is limited and easily overwhelmed. Boosting non-proteasomal, enzymatic pathways to degrade amyloid (e.g. insulin degradative enzyme) is an additional strategy as pointed out in culture studies (Schmitz *et al.* 2004) and *in vivo* (Leissring *et al.* 2003). A combined enzymatic approach may ultimately be necessary to clear amyloid and co-aggregated proteins efficiently.

The regulation of Parkin is not understood to any great degree. Parkin is induced by either mitochondrial or ER stress and in turn is cytoprotective against both insults (Bouman *et al.* 2011). These properties are seemingly independent of its intrinsic proteasome stimulatory action. Prior studies however reported that initiating the unfolded protein response (UPR) did not stimulate neuronal Parkin levels (Ledesma *et al.* 2002; West *et al.* 2003). We found that amyloid expression had a negligible effect to up-regulate endogenous Parkin but may change its solubility (Rosen *et al.* 2010). However, Parkin appears to stimulate the UPR machinery to defend cells from chemical and unfolded Pael-R induced ER stress (Takahashi and Imai 2003). Finally, not only is Parkin a mitochondrial trophin, it stimulates mitochondrial biogenesis through as yet unknown mechanisms (Kuroda *et al.* 2006).

The beneficial role of Parkin to mitigate α-synuclein and Pael-R toxicity is well documented (Petrucelli *et al.* 2002; Darios *et al.* 2003; Dawson and Dawson 2003; Yang *et al.* 2003; Lo Bianco *et al.* 2004; Yamada *et al.* 2005). In addition to its effects on β-amyloid clearance, an expanded role is further illustrated in recent works. A Parkin null strain of mouse also expressing mutated human Tau not only showed increased levels of hyperphosphorylated Tau but also amyloid deposits in the hippocampus and in certain peripheral sites. Evidence for increased oxidative stress and modifications in chaperone expression was also reported (Rodríguez-Navarro *et al.* 2008). In the presence of elevated intracellular $A\beta_{1-42}$, over-expression of Parkin diminishes pathological modification of Tau in human M17 neuroblastoma cell culture (Moussa 2009).

CHIP and $A\beta_{1-42}$ in neuronal cells

The CHIP plays dual role to maintain the native structure and homeostatic concentration of proteins. First, it acts as a co-chaperone for HSPs and second, as a ubiquitin ligase to facilitate degradation of misfolded or mutated proteins. CHIP is ubiquitously expressed throughout the cytoplasm and is structurally conserved across multiple species as well as highly expressed in brain, heart and muscle (Ballinger *et al.* 1999). CHIP's vital role in protein quality control is that it discards conformationally inflexible proteins that might otherwise overcome the chaperone system (Xu *et al.* 2008). Thus CHIP acts as a triage center that links the polypeptide binding and refolding activity of Hsp70 to the UPS (Wickner *et al.* 1999; Rosser *et al.* 2007). The attachment of CHIP to Hsp70 halts the folding of Hsp70 client proteins (Ballinger *et al.* 1999; Meacham *et al.* 2001; Younger *et al.* 2004). CHIP then assists in the ubiquitination of these substrates as they also bind to its U-box site (Jiang *et al.* 2001; Younger *et al.* 2004). The Hsp70 client proteins include proto-oncogene products, kinases, nuclear hormone receptors, and aggregation prone proteins, such as α-synuclein and Tau (McDonough and Patterson 2003; Murata *et al.* 2003; Dickey *et al.* 2007a,b).

It has been reported that CHIP plays a vital role in the metabolism of APP, as well as in the clearance of its derivative β-amyloid peptide. Through immunoprecipitation, fluorescence localization and cross-linking techniques, an interaction between CHIP and APP and CHIP and Aβ was shown (Kumar *et al.* 2007). The over-expression of CHIP both stabilizes holo-APP levels and supports the association of Aβ with ubiquitin in advance of degradation. This scenario is consistent with dual roles played by CHIP, one as a co-chaperone in reducing degradation of native protein and second, as an E3 ubiquitin ligase promoting unwanted protein degradation.

CHIP and Parkin together

There are several levels at which CHIP and Parkin may interact cooperatively to acheive protein homeostasis. CHIP dynamically participates in cell stress protection (Dai *et al.* 2003; Kampinga *et al.* 2003; Yan *et al.* 2003) for instance mitigating Pael-R toxicity. Studies involving familial PD biology reported that CHIP helps displace Hsp70 from a complex with Parkin and Pael-R, freeing Parkin to mediate Pael-R ubiquitination. CHIP may then interact with Parkin as an E4-like molecule, enhancing Parkin's E3 ligase function (Imai *et al.* 2002; Fig 3).

Aside from its name, CHIP has the added capability to selectively bind non-native substrates for ubiquitination independent of Hsp70. How this achieved is unclear. One possibility follows the interesting observation that Hsp70 itself is a novel substrate for the E3 ubiquitin ligase activity of Parkin. While elevated levels of Hsp70 are observed in the sporadic form of PD, they remain unaltered in brain tissue from Parkin-deficient ARJPD subjects (Moore *et al.* 2008). Therefore, this post-translational ubiquitin modification does not promote the degradation of Hsp70. CHIP meanwhile is shown by others to ubiquitinate both Hsc70 and Hsp70

(Jiang *et al.* 2001; Qian *et al.* 2006). The ubiquitination of HSPs by both Parkin and CHIP raises some interesting questions but its significance in the regulation of their cooperativity remains unsettled.

By numerous reports, either CHIP or Parkin E3 ligases are shown to promote proteasomal-mediated clearance of α-synuclein (Petrucelli *et al.* 2002; Hyun *et al.* 2003; Shin *et al.* 2005; Yamada *et al.* 2005) and Aβ (Kumar *et al.* 2007; Rosen *et al.* 2010; Khandelwal *et al.* 2011). In a double transgenic model expressing mutant human Tau, the deficiency of Parkin aggrevated both amyloid deposition and Tau pathology along with producing behavioral abnormalities. These changes were associated with reduced CHIP levels (Rodríguez-Navarro *et al.* 2008). However, in another model expressing human mutant APP (Swedish), the same suppression of Parkin led to improvements in these pathologies, associated with elevated CHIP/Hsp70 levels (Perucho *et al.* 2010). Thus depending on transgene context, CHIP induction can be compensatory to the loss of Parkin.

Similarly either Parkin or CHIP attenuate Tau modification and toxicity (Menéndez *et al.* 2006; Guerrero *et al.* 2008; Moussa 2009) and (Petrucelli *et al.* 2004; Dickey *et al.* 2006; Oddo *et al.* 2008), respectively. Few studies have examined CHIP and Parkin together for independency or co-operativity with respect to hyperphosphorylated Tau clearance (Shimura *et al.* 2004). Imai *et al.* (2002) showed they work together to suppress Pael-R levels. Preliminary results from our group suggest they synergize to further reduce cellular amyloid load.

Other ubiquitin E3 ligases in neurodegenerative disorders

Ubiquitin ligases have been broadly classified into three classes of E3: the homologous to E6-AP C-terminus (HECT), the RING finger, and U-box domain types. E6 protein is encoded by oncogenic strains of human papilloma virus and associates with a cellular protein termed E6-AP (E6-associated protein). E6-AP catalyzes the final attachment of ubiquitin to the tumor suppressor gene p53 in human papilloma virus-infected cells (Beer-Romero *et al.* 1997). The C-terminus of E6-AP contains the catalytic ligase domain. HECT domain family members directly ligate ubiquitin to substrate proteins. The interaction between the HECT E3 domain and target protein results in transfer of a ubiquitin molecule. Failure to express E6-AP (encoded by the UBE3A gene) in humans causes a genetic neurological disorder, Angelman Syndrome. It arises from a maternal UBE3A gene mutation or deletion and normal paternal imprinting. Although it is not a neurodegenerative disorder, children are developmentally delayed and hippocampal learning and long-term potentiation (LTP) are affected in the rodent model.

By contrast, RING-finger and U-box E3s act as 'facilitators of interaction' between an E2 and target protein, which results in direct transfer of ubiquitin from the E2 to the target protein (Ardley *et al.* 2001, 2003). There is structural homology between RING finger and U-box domains (Hatakeyama *et al.* 2001). Another domain present in many E3s is termed the WW domain. The WW domain-containing E3s, such as 'neural precursor cell expressed developmentally down-regulated' (Nedd4), usually also contain a C2 domain. The C2 domain acts in response to elevations in intracellular Ca^{2+} to promote translocation of Nedd4 to the plasma membrane (Plant *et al.* 2009). The presence of this domain in neuronal HECT E3s is essential for ligating ubiquitin to membrane bound neurotransmitter receptors and associated proteins (Glickman and Ciechanover 2002; Hegde 2004). Nedd4 is important to normal neural differentiation as well in the regulation of synaptic plasticity. The AMPA-receptor is one of several such targets of Nedd4-catalyzed ubiquitination (Schwarz *et al.* 2010). A proposed mechanism for the synaptic failure in AD is abnormal increased AMPA-receptor endocytosis and long-term synaptic depression (LTD) induction (Hsieh *et al.* 2006). Whether AMPA-R ubiquitination by Nedd4 is responsible for its internalization and trafficking to the lysosome in AD remains to be established.

RING finger E3s can be subcategorized into two types. The first type is based upon a single key subunit. N-recognin or E3 has been found to select proteins for degradation on the basis of their amino acid composition at the N-terminus (Madura *et al.* 1993; Varshavsky *et al.* 1998). Another single subunit RING finger E3 is Mdm2. In neurons Mdm2 appears to ubiquitinate the post-synaptic density protein PSD-95 (Colledge *et al.* 2003). It is best known as a negative regulator of p53. Disruption of Mdm2-p53 interactions leads to hyperphosphorylation of Tau and has consequences in other neurodegenerative models. For instance, in an animal model of PD, Mdm2 deficiency aggravates apoptotic death of dopaminergic cells (BreTaud *et al.* 2007). The second type of E3s is multi-subunit complexes and an example of this type is the modular SCF (Skp1/Cullin/F-box/ Rbx1/2) family of E3s which probably form the largest group (Deshaies 1999; Zheng *et al.* 2002). SCF (Fbx2) is shown to degrade β site APP-cleaving enzyme 1 and reduce β-amyloidogenesis (Gong *et al.* 2010).

MARCH5 is a prototype E3 ubiquitin ligase that is resident in the mitochondria. There, it binds proteins that control fission and fusion dynamics. Examples of MARCH5 clients are mitochondrial fission 1 protein (hFis1), dynamin-related protein 1 (Drp1) and mitofusin 2 (Mfn2) (Park *et al.* 2010). How these interactions regulate mitochondrial function in the context of neurodegeneration is speculative. However, there is mounting evidence for an imbalance between fusion and fission in AD, especially as pertain to DRP1 and β-amyloid (Nakamura *et al.* 2010; Santos *et al.* 2010; Manczak *et al.* 2011).

Hydroxymethylglutaryl reductase degradation 1 (HRD1) is an ER-associated E3 ligase which is up-regulated during oxidative and ER stress. It also is shown to reduce Aβ production, perhaps as part of the UPR. These and other E3

Table 1 A consolidated list of various Ubiquitin E3 ligases that actively participate in neurodegenerative disorders as listed (shaded in grey); (–) indicates no reported action of that particular E3 ligase

Ubiquitin E3 ligase	AD	PD	HD	ALS	Poly Q	IBM	References
Parkin							Tsai *et al.* 2003; Rosen *et al.* 2006; Khandelwal *et al.* 2011; Kim *et al.* 2011
Murine double minute (Mdm2)						–	Rangone *et al.* 2005; Fleischer *et al.* 2006; Nair *et al.* 2006; BreTaud *et al.* 2007; Illuzzi *et al.* 2011; Proctor and Gray 2010
Anaphase-promoting complex (APC)		–	–	–		–	Almeida *et al.* 2005; Bocharova *et al.* 2008; Maestre *et al.* 2008
Peptidylprolyl isomerase (cyclophilin)-like 2 (PPIL2)		–	–	–	–	–	Espeseth *et al.* 2006; Carson *et al.* 2009
RanBP2	–		–	–	–	–	Um *et al.* 2006
STUB1		–	–	–	–	–	Zhang *et al.* 2008a,b; Tsvetkov *et al.* 2011
TOPORS	–		–	–	–	–	Shinbo *et al.* 2005
SCF (Fbx2)		–	–	–		–	Gong *et al.* 2010; Godin *et al.* 2010
HRD1			–	–		–	Yang *et al.* 2007; Kaneko *et al.* 2010; Mei and Niu 2010; Saito *et al.* 2010; Umeda *et al.* 2011
Gigaxonin		–	–	–	–	–	Ganay *et al.* 2011
ITCH		–		–	–	–	Bellomaria *et al.* 2010
Nedd4-1 (neural-precursor cell-expressed developmentally		–	–	–	–	–	Schwarz *et al.* 2010
RNF182		–	–	–	–	–	Liu *et al.* 2008
TRAF6 (tumor necrosis factor receptor-associated factor 6)		–		–		–	Babu *et al.*2005; Zucchelli *et al.* 2011
Dactylidin		–	–	–	–	–	von Rotz *et al.* 2005
TRIM9	–		–	–	–	–	Tanji *et al.* 2010
PARK2	–		–	–	–	–	Veeriah *et al.* 2010
XIAP (X-linked inhibitors of apoptosis)	–		–	–		–	Rangone *et al.* 2005; Tsang *et al.* 2009
Siah-1 (seven in absentia homologue-1)	–		–	–		–	Lee *et al.* 2008; Sroka *et al.* 2009
Dorfin	–		–		–	–	Ishigaki *et al.* 2004
Nrdp1	–		–	–		–	Yu and Zhou 2008; Mo *et al.* 2010; Durcan *et al.* 2011
NEDL1	–	–	–		–	–	Zhang *et al.* 2011
Gp78	–	–	–			–	Ying *et al.* 2009; Yang *et al.* 2010
Atrogin-1 and MuRF1	–	–	–		–		Léger *et al.* 2006; H.-Y. Lee, E. Rocknik, Q. Fu, K. Bumsup, L. Zeng, K. Walsh and H. Querfurth, unpublished results.
HERC	–	–	–		–	–	Mashimo *et al.* 2009
Rhes (Ras homolog enriched in striatum)	–	–		–		–	Subramaniam *et al.* 2009, 2010
Sel-10		–	–	–	–	–	Li *et al.* 2002
NEDD8 (neural precursor cell expressed, developmentally down-regulated 8)		–		–	–	–	Mori *et al.* 2005
CHIP						–	Jana *et al.* 2005; Miller *et al.* 2005; Kumar *et al.* 2007; Williams *et al.* 2009
Skp1	–		–	–	–	–	Fishman-Jacob *et al.* 2009

AD, Alzheimer's disease; PD, Parkinson's disease; HD, Huntington's disease; ALS, amyotrophic lateral sclerosis; Poly Q, polyglutamine; IBM, inclusion body myositis (a muscle disorder where β-amyloid playing a key role).

ligases with possible connections to the biology of neurodegeneration are listed in Table 1.

Therapeutic considerations and concluding remarks

Ubiquitin E3 ligases are rational therapeutic targets to up-regulate because of their substrate specificity. Moreover, allosteric modification of the substrate binding region may be utilized to modulate client protein affinity and control their level of ubiquitination (Upadhya *et al.* 2006). Parkin, a multifunctional E3 ligase and neuroprotective/anti-oxidative protein is one such therapeutic candidate that may offset multiple neurodegenerative pathologies (Petrucelli *et al.* 2002; Darios *et al.* 2003; Chung and Dawson 2004; Moore 2006; Um *et al.* 2010). Recently, Parkin is found to have a major new role in PINK-1 assisted removal of damaged mitochondria or mitophagy (Vives-Bauza *et al.* 2010; Springer and Kahle 2011). Parkin also has broad stimulatory effects on the proteasome (Chan *et al.* 2011). These and the action of Parkin to ubiquitinate and hasten proteasome-mediated removal of Aβ (Khandelwal and Moussa 2010; Rosen *et al.* 2010) is worth keeping in mind as complementary clearance mechanisms such as autophagy are developed for pharmacotherapy.

CHIP has a widely accepted crucial role to maintain polyQ proteins in a soluble and non-aggregated state. It too is a potential therapeutic target to consider for several other neurodegenerative diseases (Miller *et al.* 2005). Additional work on the mechanism through which CHIP can degrade oligomeric α-synuclein may lead to drugs that boost its effects in the synucleinopathies (Tetzlaff *et al.* 2008) as well as in AD where Aβ and α-synuclein may cross-seed one another. CHIP's stabilizing effect on holo-APP and reduction of Aβ generation together with its amyloid clearing property make it particularly suitable to investigate concurrent with the Parkin-directed efforts. Counteracting both α-synuclein and amyloid toxicities could lessen Tau phosphorylation (Moussa 2009).

There are likely instances of redundancy in the action to ubiquitinate-targeted proteins among the several E3 ligases. For instance, over-expression of either CHIP or Parkin enhances ubiquitination of polyglutamine-expanded ataxin-3 and lessens its cellular toxicity (Jana *et al.* 2005; Miller *et al.* 2005). In other instances, the two ligases are likely to exhibit obligatory cooperation. Thus, multidrug strategies may be necessary to achieve full control over abnormal protein load. Hsp70/90 based therapy, not discussed further here, would predictably be neuroprotective by their ATPase-dependent folding functions as well as essential to the function of E3 ligases. As this chaperone is common to several ubiquitination pathways, it may ultimately be the best universal therapeutic target (Tsai *et al.* 2003; Jana *et al.* 2005; Morishima *et al.* 2008). Another caveat is the extent to which the proteasome is impaired in AD and PD. The nature of this dysfunction may limit the ability of E3 ligase(s) therapy to eliminate unwanted proteinopathies in spite of all their other multifunctional properties. A clearer understanding of intraneuronal processes is of major importance in recognizing which specific disease-promoting steps to inhibit and the beneficial processes such as proteasome and autophagy based clearance of protein aggregates to stimulate.

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