Published in final edited form as:

Biochem Soc Trans. 2015 April ; 43(2): 269–274. doi:10.1042/BST20140321.

Activation of the E3 Ubiquitin Ligase Parkin

Thomas R. Caulfield^{1,*}, Fabienne C. Fiesel¹, and Wolfdieter Springer^{1,2,*}

¹Department of Neuroscience, Mayo Clinic Jacksonville, Florida, USA

²Mayo Graduate School, Neurobiology of Disease, Mayo Clinic Jacksonville, Florida, USA

Abstract

The PINK1/Parkin-dependent mitochondrial quality control pathway mediates the clearance of damaged organelles, but appears to be disrupted in Parkinson's disease. Upon mitochondrial stress, PINK1 activates the E3 Ubiquitin (Ub) ligase Parkin through phosphorylation of the Ub-like (UBL) domain of Parkin and of the small modifier Ub itself at a conserved residue. Recently resolved partial crystal structures of Parkin showed a 'closed', auto-inhibited conformation, consistent with its notoriously weak enzymatic activity at steady state. It has thus become clear that Parkin must undergo major structural rearrangements in order to unleash its catalytic functions. Recent published findings derived from X-ray structures and molecular modeling present a complete structural model of human Parkin at an all atom resolution. The results of the combined *in silico* simulations-based and experimental assay-based study indicates that PINK1-dependent Ser65 phosphorylation of Parkin is required for its activation and triggering of 'opening' conformations. Indeed, the obtained structures showed a sequential release of Parkin's intertwined domains and allowed docking of an Ub-charged E2 co-enzyme, which could enable its enzymatic activity. In addition, using cell-based screening, select E2 enzymes that redundantly, cooperatively, or antagonistically regulate Parkin's activation and/or enzymatic functions at different stages of the mitochondrial autophagy (mitophagy) process were identified. Other work that aims to pinpoint the particular pathogenic dysfunctions of Parkin missense mutations has been recently disseminated. Such a structure-function approach provides the basis for the dissection of Parkin's regulation and a targeted drug design to identify small molecule activators of this neuroprotective E3 Ub ligase.

Keywords

PINK1; Parkin; Parkinson's disease; ubiquitin; simulations; molecular dynamics

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease and the result of the degeneration of dopaminergic neurons within the substantia nigra pars compacta. Besides several other genetic causes, autosomal recessive PD can be caused by

*Correspondence to: **Thomas Caulfield, PhD**, Department of Neuroscience, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, USA and **Wolfdieter Springer, PhD**, Department of Neuroscience, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, USA; Tel.: +1-904-953-6129; Fax: +1-904-953-7117; caulfield.thomas@mayo.edu; springer.wolfdieter@mayo.edu.

mutations in the PTEN-induced putative kinase 1 (*PINK1*) and *PARKIN* genes [1]. Interestingly, PINK1 and Parkin protein are cooperatively functioning within the mitochondrial quality control (mitophagy) pathway [2]. The Ser/Thr phosphorylating activity of PINK1 is pivotal for the recruitment of the E3 Ubiquitin (Ub) ligase Parkin from the cytosol to depolarized mitochondria [3–6]. Parkin catalyzes the addition of Ub to various protein targets localized at the mitochondrial surface [7,8]. Interestingly, both Ub and Parkin [9–16] are phosphorylated by PINK1 at Ser65 [14,16–18]. Phosphorylation of Parkin is necessary to activate Parkin whereas phosphorylation of Ub that is attached to mitochondrial substrate proteins might help to efficiently recruit Parkin to damaged mitochondria. The addition of poly-Ub chains to the mitochondria and the subsequent recruitment of adapter proteins cluster mitochondria at perinuclear regions and initiate degradation of individual substrates proteins and of entire organelles via the proteasome or autophagy/lysosome systems, respectively [3,7,19,20]. The neuroprotective effect of the *PINK1* and *PARKIN* genes is lost with mutations in those genes, which disrupt mitophagy at different steps during the sequential process [3–6].

PINK1 is known to phosphorylate Ser65 of Parkin within the Ub-like (UBL) domain and it has been shown that activation of Parkin's enzymatic function and mitochondrial translocation are linked [14–16,21,22]. Based on the presence of characteristic Zn^{2+} coordinating domains, Parkin has long been assumed a classical Really-Interesting-New-Gene (RING)-type E3 Ub ligase. However, a novel hybrid mechanism for Ub transfer for Parkin and other members of the RING-between-RING (RBR) family was recently identified [23]. While Parkin binds the E2 co-enzyme via its RING domain, it physically receives the Ub moiety on its active center Cys431 similar to Homologous-to-the-E6-AP-Carboxyl-Terminus (HECT)-type E3 Ub ligases. Thus, E2 co-enzymes that charge Parkin with Ub play a prominent role in its activation and enzymatic functions [24].

Several recent crystal structures for Parkin indicate a 'closed' and inactive conformation coming from several intra-molecular interactions among the individual domains [25–27]. Other structural data has been critical in developing hypotheses for the mechanism of Parkin, translocation and activation, such as the 2D-NMR HSQC spectrum based studies for individual domains, mass spectrometry, and SAXS experiments [28]. These X-ray data sources were combined with the computational modeling to establish a complete structure for human Parkin at an all-atom resolution [29]. In combination with additional molecular dynamics simulations (MDS), a conformational pathway of Parkin activation could be developed. Thereby, PINK1-dependent phosphorylation at Ser65 initiated structural changes in a series of propagating motions that released Parkin from its auto-inhibitory state, allowing activation of its enzymatic activity [29].

This review is focused on select aspects of Parkin activation that arise as a consequence of phosphorylation of the UBL domain and lays out structural and functional findings as a basis for future drug discovery efforts to stimulate the neuroprotective E3 Ub ligase Parkin.

Full-length model for human Parkin protein

Using established molecular modeling methods [30–32], recent partial X-ray structures [human N-terminal truncated Parkin (PDB IDs: 4BM9 [27] and 4I1H [25]) and of its rat homolog (PDB IDs: 4K7D and 4K95 [26])] were incorporated into the generation of a first full-length structural model for human Parkin protein [29] (Figure 1A). Briefly, the N-terminal UBL domain (residues 1–76) and in particular the flexible linker (residues 77–140) had not been previously determined. Modeling of the N-terminus revealed that the linker region is comprised of two subdomains: (1) a semi-globular domain from residue 77 to 125 that appears highly dynamic and (2) a tethering loop region from residue 126 to 140 that connects to the RING0 domain [29]. Examination of the linker's secondary structural features designates following regions: (1) α -helical regions from Gly77 to Gly85 and from Arg89 to Ser108, (2) a helix loop turn from Leu112 to Ser116, (3) minor beta-roll (anti-parallel) from Val117 to Leu123 paired to the beta-strands from the UBL domain residues Phe4-Asn8 and Leu41-Phe45, and (4) random coil from Leu123 through Arg140 [29].

Based on this full-length model of human Parkin, numerous variants carrying PD-associated mutations or post-translational modifications were created to investigate structural conformers in subsequent simulation studies. At least three intra-molecular interactions contribute to the auto-inhibition of Parkin: (1) the UBL-linker region appears to act as a spring/clamp that holds Parkin in its 'closed', intertwined conformation; (2) the repressor element of Parkin (REP) blocks the E2 co-enzyme binding site in RING1; and (3) RING0 occludes the catalytic center Cys431 in RING2, resulting in a spatial separation between the active sites of E2 and Parkin by more than 50 Å. It is thus evident that Parkin must undergo major conformational rearrangements in order to gain enzymatic activity. In general, Ub ligases have the capacity to go through large-scale conformer switching in the course of their catalytic cycles that may remodel the domain interfaces [33]. The exposure of the RING domain [34,35] as a phosphorylation-dependent process and release of the auto-inhibition for RING and HECT E3 ligases have been shown [34,36].

Phosphorylation of Parkin at Ser65 induces structural changes

Ser65 of Parkin within its UBL domain localizes to a cleft formed by the UBL-linker interface. Phospho-modification of Parkin at this residue triggered an initial structural change within the vicinity (i.e. opening of the cleft) that eventually resulted in release of the N-terminus from its close contacts with RING1 and IBR domains and further propagated into greater structural changes [29]. In addition, several mutations of Ser65 were tested to imitate/block the effect of the phosphorylation, which resulted in various cleft-widening gaps that allowed increasing (phosphomimic, Glu and Asp) or decreasing (phospho-dead, Ala) aqueous solvation of the interface during MDS sampled conformational paths [29] (Figure 1B). Pocket hydration and SASA calculations of the internal pocket were determined demonstrating the pronounced effect from phosphorylation at Ser65 (Supplemental Movie S1) that were confirmed by cell-based analysis [29].

Subsequent large-scale MDS led to the generation of >30,000 conformers for a pathway describing the complete dissociation of the UBL domain from the auto-inhibited

conformation through an open and activated form of Parkin [29]. Various inter-domain parameters on the structure (RMSD, RMSF, distance based metrics) indicate that several 'safety belts' which hold Parkin in its 'closed' conformation are released prior to triggering E3 ligase activity [29]. For activation of Parkin, the following key regions are released: UBL-linker region dissociates from the RING1 and IBR domains; the REP region is loosened of its RING1 contacts (Tyr391 to Cys238 distance increases) making the E2 binding site available for association of an Ub-loaded co-enzyme; and the RING0 domain is released resulting in increased hydration around the active site residue Cys431 (Figure 1C). It is assumable that these three 'safety belts' are triggered sequentially [29].

Various MD simulations were conducted using pSer65 and Ser65 to study the motion of the auto-inhibited structure that leads to an active conformation of the E3 enzyme. The molecular motion required for release of the auto-inhibitory structure was furthered using accelerated molecular dynamics and sampling algorithms, like Maxwell's demon molecular dynamics [31,32], which allows for speeded sampling along conformation space. In the cases where the structure is between two known conformers, sampling algorithms are quite efficient and useful. A large set of conformers was generated for analysis that revealed critical interactions at the 'safety belts' to be triggered step-wise during release of the UBL domain and motion away from IBR toward RING2. Particularly of note, the generated data indicated that the REP region becomes unbound from E2 region, thus allowing an incoming E2 enzyme greater access to negotiate binding at the E2 site.

E2 co-enzyme usage of Parkin

By scoring Parkin translocation upon siRNA-mediated knock-down of specific E2 enzyme using High Content Imaging (HCI), several co-enzymes were identified that either positively or negatively regulate Parkin activation, translocation and enzymatic functions during mitochondrial quality control [24]. UBE2D family members and UBE2L3 act redundantly and are necessary for charging of Parkin with Ub and thus its translocation to damaged mitochondria. Using the HCI scoring system, also knockdown of UBE2N was also found to exhibit a minor effect on Parkin translocation to mitochondria, although it primarily affected clustering of mitochondria around perinuclear regions as determined in follow-up assays. UBE2N was not required for charging of Parkin with Ub, but rather cooperatively acted together, but downstream of UBE2D and UBE2L3. Another recent study has come to the same overall conclusion that UBE2N, UBE2D and UBE2L are required for efficient execution of Parkin-dependent mitophagy, although the authors do not attribute this effect to a delay in translocation but to the inhibition of autophagy [37]. At least for UBE2N this has been questioned recently [38]. Interestingly, knockdown of UBE2R1 enhanced Parkin translocation and increased mitochondrial clustering [24]. Although the exact molecular mechanism remained elusive, the existence of such negative regulation of Parkin by Ub signaling may open up further avenues for a targeted activation of Parkin.

A role for UBE2D and UBE2L3 for Parkin autoubiquitination had already been established [23]. Kazlauskaitė and colleagues performed a comprehensive *in vitro* ubiquitination assay with Miro1 as a substrate [17]. In addition to UBE2D and UBE2L3 several other E2 enzymes (UBE2E, UBE2H, UBE2J, UBE2K, and UBE2W) showed a much higher activity

in the presence of functional PINK1. In contrast to another study, a prominent role of UBE2A for Parkin-dependent mitophagy (Rad6a) [39] could not be established. The genome-wide siRNA screen by Hasson et al. that monitored Parkin translocation, revealed several E2 enzymes [40]. In addition to UBE2D and UBE2L3, the endoplasmic reticulum (ER)-anchored UBE2J2/NCUBE2 that has been implicated in ER-associated degradation (ERAD) pathway and the orphan E2 UBE2O/E2-230K were identified. It is thus possible that additional E2 enzymes play important roles during Parkin-mediated mitophagy. In fact, it seems that specific E2 enzymes operate at distinct steps during this process and likely with different purposes.

Effect of PD mutations on Parkin structure and function

Recent work from Springer and co-workers (Fiesel *et al.*, submitted) examined 21 *PARKIN* missense mutations distributed across all individual domains of Parkin, including K161N, K211N, T240R, R275W and G430D that were modeled on phospho-Ser65 Parkin protein structure (Figure 1A). In order to pinpoint the exact dysfunctions of individual Parkin mutations during the sequential process of Parkin activation and mitophagy, local and global conformational changes that occur in the newly published structural model using MDS [29] were examined in addition to functional defects. Findings from this study indicate that e.g. K161N and K211N only minimally induce conformational change. Pathogenic effect of these mutations is likely to be the result of mutating the positively charged residues to uncharged asparagine that may lower the affinity of a presumed phospho-binding site in RING0 to incoming phospho-Ub moieties. In contrast, T240R located in the E2 binding site showed major local conformational changes that likely result in stabilization of the REP region with the adjacent domains via an arginine-to-REP region interaction (Fiesel *et al.*, submitted), which likely disrupts E2 binding (Figure 2A–B). The R275W mutation created a swatch of local and global conformational changes that propagate throughout the entire structure (Fiesel *et al.*, submitted). The G430D mutation only seemed to alter the active site by delocalizing the attractive charge for Cys431, which would consequently lower the catalytic efficiency for the reaction with substrates. HCI analysis to measure Parkin translocation to damaged mitochondria and complementary enzymatic activity measurements (specifically Ub-charging experiments with and without CCCP treatment) were used to demonstrate agreement within the dataset (Fiesel *et al.*, submitted).

Concluding remarks

Parkin is an auto-inhibited Ub E3 ligase with roles in the regulation of mitochondrial quality control mediated by both proteasome and autophagy/lysosome degradation systems. While Parkin acts broadly neuroprotective, inactivation/inhibition through mutations or post-translational modifications results in PD. Given the complex and sequential activation process of Parkin that involves phosphorylation and consequent structural changes, several distinct dysfunctions can result in its loss of function. Structure-function studies now provide the chance not only to dissect the regulation and enzymatic activity of Parkin, but also to identify chemical and genetic modifiers that may be useful to activate Parkin and/or to maintain an active conformation. Current work is underway to identify, discover, or design small molecules that opportunistically take advantage of the 'safety belts' critical for

Parkin's auto-inhibition. The key determinant going forward will be a complete understanding of Parkin's activation and the ability to develop efficacy, ADME, and improved target specificity with cell-based and *in vivo* experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by the National Institutes of Health/National Institute of Neurological Disorders And Stroke [R01NS085070], Michael J. Fox Foundation for Parkinson's Research, Foundation for Mitochondrial Medicine, Mayo Clinic Foundation and the Center for Individualized Medicine (CIM), Marriott Family Foundation, and a Gerstner Family Career Development Award to W.S.

Abbreviations

PD	Parkinson's disease
PINK1	PTEN-induced putative kinase 1
Ub	Ubiquitin
UBL	Ub-like (domain)
RING	Really-Interesting-New-Gene
IBR	in-between-RING
RBR	RING-between-RING
HECT	Homologous-to-the-E6-AP-Carboxyl-Terminus
RMS	root-mean-square
RMSF	root-mean-square-fluctuation
RMSD	root-mean-square-deviation, MDS, molecular dynamics simulation
MC	Monte Carlo algorithm
VS	virtual screening
HCI	High Content Imaging

References

1. Corti O, Lesage S, Brice A. What genetics tells us about the causes and mechanisms of Parkinson's disease. *Physiological reviews*. 2011; 91:1161–1218. [PubMed: 22013209]
2. Springer W, Kahle PJ. Regulation of PINK1-Parkin-mediated mitophagy. *Autophagy*. 2011; 7:266–278. [PubMed: 21187721]
3. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nature cell biology*. 2010; 12:119–131. [PubMed: 20098416]

4. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, et al. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *The Journal of cell biology*. 2010; 189:211–221. [PubMed: 20404107]
5. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS biology*. 2010; 8:e1000298. [PubMed: 20126261]
6. Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, et al. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:378–383. [PubMed: 19966284]
7. Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, et al. Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Human molecular genetics*. 2011; 20:1726–1737. [PubMed: 21296869]
8. Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, et al. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature*. 2013; 496:372–376. [PubMed: 23503661]
9. Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, et al. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *The Journal of cell biology*. 2014; 205:143–153. [PubMed: 24751536]
10. Kazlauskaitė A, Kondapalli C, Gourlay R, Campbell DG, Ritorto MS, et al. Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *The Biochemical journal*. 2014; 460:127–139. [PubMed: 24660806]
11. Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, et al. Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature*. 2014; 510:162–166. [PubMed: 24784582]
12. Ordureau A, Sarraf SA, Duda DM, Heo JM, Jedrychowski MP, et al. Quantitative Proteomics Reveal a Feedforward Mechanism for Mitochondrial PARKIN Translocation and Ubiquitin Chain Synthesis. *Molecular cell*. 2014; 10.1016/j.molcel.2014.09.007
13. Zhang C, Lee S, Peng Y, Bunker E, Giaime E, et al. PINK1 Triggers Autocatalytic Activation of Parkin to Specify Cell Fate Decisions. *Current biology : CB*. 2014; 10.1016/j.cub.2014.07.014
14. Kondapalli C, Kazlauskaitė A, Zhang N, Woodroof HI, Campbell DG, et al. PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open biology*. 2012; 2:120080. [PubMed: 22724072]
15. Shiba-Fukushima K, Imai Y, Yoshida S, Ishihama Y, Kanao T, et al. PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes mitochondrial translocation of Parkin and regulates mitophagy. *Scientific reports*. 2012; 2:1002. [PubMed: 23256036]
16. Iguchi M, Kujuro Y, Okatsu K, Koyano F, Kosako H, et al. Parkin-catalyzed ubiquitin-ester transfer is triggered by PINK1-dependent phosphorylation. *The Journal of biological chemistry*. 2013; 288:22019–22032. [PubMed: 23754282]
17. Kazlauskaitė A, Kelly V, Johnson C, Baillie C, Hastie CJ, et al. Phosphorylation of Parkin at Serine65 is essential for activation: elaboration of a Miro1 substrate-based assay of Parkin E3 ligase activity. *Open biology*. 2014; 4:130213. [PubMed: 24647965]
18. Sauve V, Gehring K. Phosphorylated ubiquitin: a new shade of PINK1 in Parkin activation. *Cell research*. 2014; 24:1025–1026. [PubMed: 24946738]
19. Okatsu K, Saisho K, Shimanuki M, Nakada K, Shitara H, et al. p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. *Genes to cells : devoted to molecular & cellular mechanisms*. 2010; 15:887–900. [PubMed: 20604804]
20. Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, et al. Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *The Journal of cell biology*. 2010; 191:1367–1380. [PubMed: 21173115]
21. Lazarou M, Narendra DP, Jin SM, Tekle E, Banerjee S, et al. PINK1 drives Parkin self-association and HECT-like E3 activity upstream of mitochondrial binding. *The Journal of cell biology*. 2013; 200:163–172. [PubMed: 23319602]
22. Zheng X, Hunter T. Parkin mitochondrial translocation is achieved through a novel catalytic activity coupled mechanism. *Cell research*. 2013; 23:886–897. [PubMed: 23670163]
23. Wenzel DM, Lissounov A, Brzovic PS, Klevit RE. UBC7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. *Nature*. 2011; 474:105–108. [PubMed: 21532592]

24. Fiesel FC, Moussaoud-Lamodiere EL, Ando M, Springer W. A specific subset of E2 ubiquitin-conjugating enzymes regulate Parkin activation and mitophagy differently. *J Cell Sci.* 2014; 127:3488–3504. [PubMed: 24928900]
25. Riley BE, Loughheed JC, Callaway K, Velasquez M, Brecht E, et al. Structure and function of Parkin E3 ubiquitin ligase reveals aspects of RING and HECT ligases. *Nature communications.* 2013; 4:1982.
26. Trempe JF, Sauve V, Grenier K, Seirafi M, Tang MY, et al. Structure of Parkin Reveals Mechanisms for Ubiquitin Ligase Activation. *Science.* 2013; 340:1451–1455. [PubMed: 23661642]
27. Wauer T, Komander D. Structure of the human Parkin ligase domain in an autoinhibited state. *The EMBO journal.* 2013; 32:2099–2112. [PubMed: 23727886]
28. Spratt DE, Julio Martinez-Torres R, Noh YJ, Mercier P, Manczyk N, et al. A molecular explanation for the recessive nature of parkin-linked Parkinson's disease. *Nature communications.* 2013; 4:1983.
29. Caulfield TR, Fiesel FC, Moussaoud-Lamodiere EL, Dourado DF, Flores SC, et al. Phosphorylation by PINK1 Releases the UBL Domain and Initializes the Conformational Opening of the E3 Ubiquitin Ligase Parkin. *PLoS computational biology.* 2014; 10:e1003935. [PubMed: 25375667]
30. Zhang YJ, Caulfield T, Xu YF, Gendron TF, Hubbard J, et al. The dual functions of the extreme N-terminus of TDP-43 in regulating its biological activity and inclusion formation. *Human molecular genetics.* 2013; 22:3112–3122. [PubMed: 23575225]
31. Caulfield T, Devkota B. Motion of transfer RNA from the A/T state into the A-site using docking and simulations. *Proteins.* 2012; 80:2489–2500. [PubMed: 22730134]
32. Caulfield TR, Devkota B, Rollins GC. Examinations of tRNA Range of Motion Using Simulations of Cryo-EM Microscopy and X-Ray Data. *J Biophys.* 2011; 2011:219515. [PubMed: 21716650]
33. Lorenz S, Cantor AJ, Rape M, Kuriyan J. Macromolecular juggling by ubiquitylation enzymes. *BMC biology.* 2013; 11:65. [PubMed: 23800009]
34. Kales SC, Ryan PE, Lipkowitz S. Cbl exposes its RING finger. *Nature structural & molecular biology.* 2012; 19:131–133.
35. Dou H, Buetow L, Hock A, Sibbet GJ, Vousden KH, et al. Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. *Nature structural & molecular biology.* 2012; 19:184–192.
36. Gallagher E, Gao M, Liu YC, Karin M. Activation of the E3 ubiquitin ligase Itch through a phosphorylation-induced conformational change. *Proceedings of the National Academy of Sciences of the United States of America.* 2006; 103:1717–1722. [PubMed: 16446428]
37. Geisler S, Vollmer S, Golombek S, Kahle PJ. The ubiquitin-conjugating enzymes UBE2N, UBE2L3 and UBE2D2/3 are essential for Parkin-dependent mitophagy. *J Cell Sci.* 2014; 127:3280–3293. [PubMed: 24906799]
38. Shiba-Fukushima K, Inoshita T, Hattori N, Imai Y. Lysine 63-linked Polyubiquitination Is Dispensable for Parkin-mediated Mitophagy. *The Journal of biological chemistry.* 2014; 289:33131–33136. [PubMed: 25336644]
39. Haddad DM, Vilain S, Vos M, Esposito G, Matta S, et al. Mutations in the intellectual disability gene Ube2a cause neuronal dysfunction and impair parkin-dependent mitophagy. *Molecular cell.* 2013; 50:831–843. [PubMed: 23685073]
40. Hasson SA, Kane LA, Yamano K, Huang CH, Sliter DA, et al. High-content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy. *Nature.* 2013; 504:291–295. [PubMed: 24270810]

Figure 1. Structural modeling of full-length human Parkin protein and MDS studies

A. Full-length structural model for human Parkin protein [29]. The 3D structural domains are color matched to with UBL in red, linker in gray, Ring0 in green, Ring1 in cyan, IBR in purple, REP in yellow, and Ring2 in pink. Structure is rendered in ribbon-cartoon with semi-transparent solvent-accessible surface-area overlaid to illustrate protein bulk structure. Each domain and discussed mutant residue is labeled. **B.** Critical effect of phosphorylation at Serine-65 is shown for Parkin structure. Left panel is early in MDS sampling and right panel is after >120 ns of MDS. The effect on the cleft between the linker and UBL domains is shown. **C.** Mutation in the active site region for Parkin is shown. Nearby residues (<4Å) that may alter binding are shown in licorice rendering. The zinc finger ions are shown as cyan

spheres. Mutation of G430D alters electrostatic distribution for the adjacent catalytic center Cys431.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Molecular dynamics effect on Parkin REP region from T240R mutation

A. Initial orientation of Arg240 is shown pointed away from REP into aqueous. Rendering is shown in licorice (stick) with coloring in standard (Carbon-gray, Oxygen-red, Nitrogen-blue, Sulfur-yellow). For reference, original position for T240 is shown in Goodsell style. **B.** After 120 ns of MDS, the Arg240 residue engages in repeated interactions with residues from REP. Rendering same as in A.