pUBLically unzipping Parkin: how phosphorylation exposes a ligase bit by bit

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Disposal of damaged mitochondria is tightly controlled by auto-inhibition mechanisms that keep the ubiquitin ligase Parkin in check. Several new structural studies provide insight into how PINK1-dependent phosphorylation of ubiquitin and Parkin may progressively relieve Parkin auto-inhibition.

A ccumulation of damaged mitochondria is prevented by their disposal through mitophagy, a mitochondrial quality control pathway. The protein kinase PINK1 and the E3 ubiquitin (Ub) ligase Parkin cooperate to initiate mitophagy by selective ubiquitination of protein substrates located on the mitochondrial outer membrane. Function-compromising mutations in PINK1 and Parkin genes predispose to hereditary early-onset Parkinson’s disease, highlighting the importance of this process.

As the sensor of mitochondrial damage, PINK1 induces Parkin translocation to mitochondria and its subsequent activation to promote ubiquitination of mitochondrial proteins and initiate mitophagy (Eiyama & Okamoto, 2015; Pickrell & Youle, 2015). Under basal conditions, ubiquitin ligase activity of Parkin is suppressed through auto-inhibitory intramolecular interactions, with all individual Parkin domains engaging in a tight embrace. Earlier structural studies of Parkin in this compact, inactive state (Riley et al., 2013; Trempe et al., 2013; Wauer & Komander, 2013) revealed that the ubiquitin-like (UbI) domain and a small “repressor” (REP) element bind to the putative ubiquitin-conjugating enzyme (E2) binding site on RING1 (R1), and interactions between RING0 (R0) and RING2 (R2) partially occlude the active site located in the R2 domain. Therefore, major rearrangements would have to take place to allow binding of ubiquitin-loaded E2 (E2~Ub) and subsequent transfer of ubiquitin onto the Parkin active-site cysteine.

Early studies of Parkin activation demonstrated that its N-terminal UbI domain has an auto-inhibitory function, with its removal increasing Parkin’s ligase activity (Chaugule et al., 2011). Furthermore, UbI domain phosphorylation on Ser65 by PINK1 was also found to stimulate Parkin (Kondapalli et al., 2012; Shiba-Fukushima et al., 2012); still, the underlying mechanism of UbI-dependent activation remained elusive. Last year, another piece of the puzzle came from several reports showing that PINK1 also phosphorylates ubiquitin itself and that non-covalent binding of phospho-Ub (pUb) to Parkin stimulates catalytic activity as well (Kane et al., 2014; Kazlauskaite et al., 2014; Koyano et al., 2014; Ordureau et al., 2014). How removal of the UbI, phosphorylation of Parkin’s UbI (pParkin), and/or binding of pUb might alter the multiple intramolecular interactions defining the auto-inhibited state, and promote an active conformation able to bind E2~Ub and transfer ubiquitin onto the active-site cysteine was however still unclear.

Now, three reports provide a first glimpse of how Parkin’s ligase activity is stimulated (Kumar et al., 2015; Sauvé et al., 2015; Wauer et al., 2015). High-resolution structures of “full-length” Parkin (here, “full-length” refers to Parkin containing all core domains UbI-R0-R1-IBR-REP-R2, but a shortened UbI-R0 linker) reveal atomic-level detail of how the UbI domain contacts other domains within the compact, inactive form of Parkin (Fig 1A). Additionally, two structures of more activated forms of Parkin have been solved, one in which pUb is bound to a Parkin version lacking only its UbI domain (ΔUbI), and a “full-length” Parkin version containing a phospho-mimetic mutation in the UbI domain (S65D-UbI). Remarkably, structural superposition of fully inhibited Parkin onto the more active forms does not reveal the anticipated rearrangement of Parkin domains relative to each other. In particular, neither of the key auto-inhibitory elements REP and R0 occupy altered positions in the new structures (Fig 1A and B). Furthermore, the UbI domain and pUb both bind to R1 but on opposite and non-overlapping surfaces (Fig 1B), yet biochemical data show that pUb binding releases the UbI (Kazlauskaite et al., 2015; Kumar et al., 2015; Sauvé et al., 2015). So how does pUb binding affect UbI binding?

Here, the new pUb-bound ΔUbI-Parkin structure shows that the IBR domain undergoes a large movement upon pUb recognition, through a lever motion caused by straightening of a kinked helix connecting R1 and IBR (Fig 1B). Examination of the new “full-length” structures reveals that in addition to its major interaction with R1, the UbI domain also contacts the IBR domain and the REP element (Kumar et al., 2015; Sauvé et al., 2015). IBR rotation as observed in the pUb-bound ΔUbI-Parkin structure would disrupt the UbI/IBR contacts, thereby weakening UbI association. Furthermore, IBR rotation stretches the REP-linker fragment and moves the linker away from the UbI domain (Wauer et al., 2015). Nevertheless, exactly how movement of the IBR...
domain might lead to Parkin activation cannot be explained by the pUb-bound ΔUbl-Parkin structure alone. But combining structural comparison of the “full-length” structures with the pUb-bound ΔUbl-Parkin structure, as well as with the extensive biochemical data included in the new reports, provides important mechanistic clues regarding how pUb binding-induced IBR rotation might lead to Parkin activation.

Ubl release increases the accessibility of Ubl residue Ser65 for modification by PINK1, as shown by in vitro phosphorylation assays—an effect that can be recapitulated by R1 mutations that weaken the Ubl: R1 interaction (Kazlauskaite et al., 2015; Sauvé et al., 2015; Wauer et al., 2015). Ubl phosphorylation decreases its affinity for Parkin, implying that once phosphorylated, the Ubl will not readily re-associate with Parkin, thereby maintaining an active state. Moreover, phosphorylation of the Ubl is associated with increased affinity between pUb and pParkin, likely due to an allosteric pUb-induced remodeling of the pUb binding site on R1 as observed in the phosphomimetic S65D-Parkin structure (Kumar et al., 2015). The reciprocal feedback relationship of pUb and pUbl makes it difficult to establish a temporal order of events. Does Parkin binding to pUb on mitochondria lead to the displacement of its Ubl domain to allow its phosphorylation by PINK1 and therefore activation? Or, does Parkin phosphorylation by PINK1 promote a tighter association with pUb to stabilize Parkin on mitochondria? It is conceivable that both events occur and that low levels of pUb and pParkin can initiate a feedback loop between the two activators.

Many questions remain regarding how pUb and pUbl activate Parkin’s catalytic activity. Although a full understanding remains out of reach, the new studies provide important insights. Auto-inhibited Parkin does not readily bind E2s, so an essential aspect of activation is overcoming this barrier. Phosphorylation of Parkin increases its affinity for the E2 UbcH7, as does binding of pUb (Kumar et al., 2015; Ordureau et al., 2015; Sauvé et al., 2015). How is enhanced E2 binding brought about? As mentioned earlier, structures of auto-inhibited Parkin have the putative E2 binding site on R1 occluded by the REP element and current models predict that displacement of this element is required for activation. Although the REP remains out of reach, the new studies provide important insights.
observed in the pUb-bound structure and an inferred extension of the IBR-REP linker hint at how the activation process might reposition the REP to facilitate E2 binding. Furthermore, data from Kumar et al (2015) suggest that ubiquitin arriving in the form of E2–Ub might increase affinity for Parkin by binding R1 at a site close to that occupied by Ubl in the inhibited state (Kumar et al, 2015; Sauvé et al, 2015). Consistent with this notion, pParkin with its Ubl domain disengaged has a higher affinity for UbcH7–Ub than for unloaded UbcH7, suggesting that the conjugated Ub contributes to the binding energy.

It should also be noted that a flexible linker connects R2 to the REP element and that R0 is connected by a flexible linker to the Ubl domain, providing a way to link movement of either the Ubl or REP to movement of the R0/R2 unit, which could potentially lead to further activation. Based on the intertwined nature of all five Parkin domains, it is tempting to speculate that disruption of contacts between some domains may trigger movements of other domains. However, additional studies are required to support this speculation, especially as none of the predicted domain rearrangements are observed in the Parkin structures solved to date.

The progress made by many dedicated researchers provides a significant step forward in our understanding of the mechanism surrounding Parkin’s ligase (in)activity. We now have a better understanding of how phosphorylation of both the Ubl and Ub increases Parkin’s ligase activity, and we have learned more about structural changes that take place upon activation. Yet, several central mysteries remain. We still do not have a clear picture of the fully activated state of Parkin. The remarkable similarities among structures of auto-inhibited Parkin and more active forms suggest that either (1) Parkin activation comes about through (multiple) small changes or (2) active conformations have not yet been captured in the existing crystal structures. So far, attempts to crystallize pParkin have not been successful hinting at its potentially more dynamic nature. Likely, the presence of additional components such as an E2–Ub conjugate and/or substrate will be required to reveal what truly active Parkin looks like. This is no small order and will require some new strategies. In the meantime, the new studies discussed here provide important contributions and insights into the fascinating and biologically critical question of how a kinase (PINK1) and a ligase (Parkin) work together to initiate mitophagy.

References