Policing Parkin with a UbID

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Mutations in the E3 ubiquitin ligase Parkin are linked to Autosomal Recessive Juvenile Parkinsonism, including a cluster of pathogenic mutations in exon 2 that gives rise to Parkin's N-terminal ubiquitin-like domain (UbID). A study in this issue of *The EMBO Journal* reveals a new role for this domain, showing that the UbID engages in an intramolecular interaction with Parkin's C-terminal region to restrict autoubiquitination. Loss of this autoinhibitory mechanism in pathogenic variants renders Parkin constitutively active. Moreover, other proteins that bind to Parkin's UbID can also relieve autoinhibition, suggesting control by substrate-mediated activation.

Post-translational modification by ubiquitin occurs through an enzymatic cascade that typically culminates in isopeptide bond formation between ubiquitin's C-terminus and a substrate lysine. After initial activation by an E1 activating enzyme, ubiquitin is passed on via thioester relay to the catalytic cysteine of an E2 conjugating enzyme; a third class of enzyme, E3 ubiquitin ligases, binds both ubiquitin-carrying E2s and the substrate of ubiquitination. Transfer of ubiquitin from an E2 to a protein substrate can occur directly, with the E3 acting only as a scaffold—as in the case of RING/U-box E3s; or by passage of activated ubiquitin via a catalytic cysteine of the E3, as in the case of HECT domain E3s.

Various mechanisms control E3 enzymes to prevent unbridled ubiquitination: post-translational modifications can alter E3 affinity for specific E2s or substrates, induce conformational changes that modulate E3 activity, or change E3 subcellular localization. Another mechanism found in the cases of the N-end rule RING-E3 Ubr1 and the C2-WW-HECT-domain E3 ligase Smurf2 is autoinhibitory restraint through intramolecular interactions. Ubr1 has a C-terminal autoinhibitory domain that restricts access to N-terminal substrate-binding sites (Du *et al*, 2002), whereas Smurf2's C2 domain binds its C-terminal HECT domain to interfere with ubiquitin thioester formation (Wiesner *et al*, 2007).

The study from the Walden group (Chaugule *et al*, 2011) in this issue reveals a novel autoregulatory mechanism for the E3 ligase Parkin, whereby its UbID is used to restrict its activity. Parkin belongs to the RING-in-between-RING (RBR) family of E3 ubiquitin ligases characterized by two RING domains separated by an 'in-between-RING-fingers' domain. It also contains an N-terminal UbID, which has been demonstrated to interact with multiple proteins in distinct pathways: an interaction with ubiquitin-interacting motifs (UIMs) in Eps15, allowing Parkin-mediated Eps15 ubiquitination, has

implicated it in EGF receptor trafficking and PI3K-Akt signaling (Fallon *et al*, 2006); it further binds SH3 domains from endocytic BAR proteins such as endophilin-A, thus linking Parkin to synaptic ubiquitination (Trempe *et al*, 2009). Parkin was likewise reported to bind the proteasome subunit S5a/Rpn10 via the UbID (Sakata *et al*, 2003), and to activate the 26S proteasome by enhancing interaction between its 19S components (Um *et al*, 2010). Parkin is also linked to mitochondrial quality control (Narendra *et al*, 2008), and the UbID promotes Parkin recruitment to, and subsequent mitophagy of, depolarized mitochondria (Narendra *et al*, 2010).

Chaugule *et al* (2011) now reveal an unexpected new intramolecular interaction for Parkin's UbID. They identify a novel UbID-binding site (the 'PUB' site) that maps to Parkin's C-terminal portion and interacts in an intramolecular manner with the UbID to prevent Parkin autoubiquitination. Loss of this interaction by UbID deletion or mutation, or even by N-terminal Parkin tagging leads to constitutive Parkin autoubiquitination; the latter observation explaining discrepant earlier reports on Parkin being constitutively auto-ubiquitinated. Moreover, all four tested disease mutations

Figure 1 Model of Parkin regulation by autoinhibition. Parkin is newly classified as RING/HECT-hybrid E3 ligase, with cysteine C431 proposed to form a thioester ubiquitin intermediate *en route* to substrate ubiquitination. The N-terminal ubiquitin-binding domain (UbID) binds to a C-terminal UbID-binding (PUB) site to block interaction with ubiquitin, restricting E2 ~ ubiquitin thioester discharge and Parkin autoubiquitination. This autoinhibition is lost in pathogenic *parkin* protein products with UbID mutations.

in the UblD also resulted in loss of the autoinhibitory mechanism and yielded constitutively ubiquitinated Parkin, thus offering context to the effects of pathogenic *parkin* products. Consistently, decreased cellular protein levels were reported for some of these mutants (Henn *et al*, 2005), although whether they resulted from constitutive autoubiquitination followed by proteasomal degradation or from an independent cellular quality control mechanism remains to be established.

Physiologically, Parkin autoinhibition is likely to be relieved by its interaction with substrates, as Chaugule *et al* demonstrate that both Eps15 UIMs and endophilin-A SH3 domain can activate Parkin autoubiquitination. Hence, disruption of UblD–PUB interaction by UblD binding to effectors or substrates can generate an equivalent catalytic outcome as UblD loss. In this respect, one caveat to the present study is that ubiquitin conjugation to a non-self substrate was not studied, and whether autoubiquitination is representative of Parkin-mediated substrate ubiquitination remains unclear.

Additional insight into this matter is provided by another new study on Parkin and the related E3 HHARI, which reveals these RBR family members to function as RING/HECT hybrids (Wenzel *et al*, 2011). Despite lacking obvious HECT domains, Parkin RING1-IBR-RING2 fragments undergo autoubiquitination in the presence of the HECT-preferring E2 UbcH7, requiring a cysteine residue conserved among RBR family members. Although no ubiquitin adduct at Parkin's Cys431 was found (even when cysteine was replaced by serine to yield a more stable ester adduct), serine substitution of the equivalent cysteine in HHARI indeed allowed detection of an adduct with ubiquitin (Wenzel *et al*, 2011). This indicates that RBR E3s such as Parkin perform catalysis in a HECT-type manner, possibly for both autoubiquitination and substrate ubiquitination.

The results of Chaugule *et al* are consistent with Parkin's catalytic role extending beyond canonical RING-type scaffolding, as they find productive autoubiquitination to require direct Parkin–ubiquitin interaction. As ubiquitin-binding and UblD-binding sites overlap, the authors propose that the UblD inhibits autoubiquitination by sterically hindering ubiquitin

binding to Parkin's C-terminal catalytic portion (Figure 1). Indeed, only Parkin variants with compromised UblD–PUB site interaction, but not wild-type Parkin, were found to stimulate E2~ubiquitin thioester discharge *in vitro*.

Taken together, a compelling model is that the UblD prevents C431~ubiquitin thioester formation, which may be a prerequisite to both autoubiquitination and non-self ubiquitination. However, further studies are needed to fully decipher the regulatory role of Parkin's UblD. Does it indeed prevent thioester bond formation with ubiquitin? and importantly, is a Parkin–ubiquitin thioester also formed during catalysis with RING-supporting E2s such as UbcH5? Recent work on a Nedd4 E3 HECT:ubiquitin complex elegantly demonstrated that Nedd4's ubiquitin-binding surface promotes substrate polyubiquitination by binding a substrate-attached ubiquitin moiety (Maspero *et al*, 2011). While loss of this interaction prevented non-self ubiquitination, Nedd4 autoubiquitination was, however, not impaired, arguing for caution when comparing *trans*- and *cis*-ubiquitination. Thus, restricting Parkin autoubiquitination via the UblD might in fact serve to activate it towards a substrate, by preventing misdirected decoy activity or by protecting Parkin from ubiquitin-dependent proteasomal degradation in the cell.

Finally, the UblD–PUB site interaction may also be regulated by additional mechanisms, such as phosphorylation. Several protein kinases can phosphorylate Parkin, and in the brain, phosphorylation-favouring conditions were found to enhance Parkin binding to endophilin-A1 (Trempe *et al*, 2009). Perhaps Parkin phosphorylation at specific locations may reduce UblD–PUB site affinity and thereby promote interaction with substrates. Clearly, the new and unexpected conformational switch within Parkin paves the way for further studies into the regulatory mechanisms activating this important E3 ligase only when needed, and otherwise keeping it in check.

Conflict of interest

The authors declare that they have no conflict of interest.

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