Compact Parkin only: insights into the structure of an autoinhibited ubiquitin ligase

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Mutations in Parkin represent ~50% of disease-causing defects in autosomal recessive-juvenile onset Parkinson's disease (AR-JP). Recently, there have been four structural reports of autoinhibited forms of this RING-IBR-RING (RBR) ubiquitin ligase (E3) by the Gehring, Komander, Johnston and Shaw groups. The important advances from these studies set the stage for the next steps in understanding the molecular basis for Parkinson's disease (PD).

Regulated protein degradation requires that E3s and their access to substrates be exquisitely controlled. RBR family E3s provide striking examples of this regulation. The complex and compact structures of Parkin (Riley *et al*, 2013; Spratt *et al*, 2013; Trempe *et al*, 2013; Wauer and Komander, 2013) as well as another RBR E3, human homologue of Ariadne (HHARI) (Duda *et al*, 2013), demonstrate extraordinarily intricate inter-domain arrangements. These autoinhibited structures ensure that their functions are restricted until activated.

Until recently, RBR E3s were believed to be a subclass of RING E3s, which allosterically activate E2 conjugated with ubiquitin (E2~Ub). However, Wenzel *et al* (2011) determined that they are actually hybrid E3s, containing an E2 binding site in RING1 and a catalytic cysteine residue in the domain designated as RING2. The catalytic cysteine is an acceptor for an ubiquitin from RING1-bound E2~Ub forming an intermediate (E3~Ub) that leads to substrate or autoubiquitination. In this way, RBRs resemble HECT E3s, which also form catalytic intermediates in ubiquitination. There are 13 human RBR family E3s. Besides Parkin, two notable RBRs are HOIL-1 and HOIP, which form part of a complex integral to NF- κ B activation (Wenzel and Klevit, 2012).

In addition to causal roles in AR-JP, single allele mutations of Parkin are found in some sporadic cases of PD (references in Wauer and Komander, 2013). Mutations in the Parkin-

Figure 1 Schematic and spatial representation of Parkin. (A) Primary structure and domain designations of Parkin, including the REP sequence within the otherwise unstructured IBR-RING2 linker. (B) Structural representation of full-length Parkin (PDB 4K95) highlighting the complex domain interactions in the three-dimensional structure, the catalytic C431 residue, and residue W403 within the REP, which plays a role in stabilizing the autoinhibited form of Parkin. (C) A model of Parkin with the E2 UbH5B/Ube2D2 bound (devised using PDB 4K95 and PDB 4AP4 to mimic the position of an E2 bound to RING1) to illustrate the required displacement of UbLD and REP and the large distance between the E2~Ub attachment site of the E2 and the catalytic active site of Parkin. Note that in this conformation the catalytic Cys within RING2 (C431) remains buried by RING0.

associated kinase PINK1, which is upstream of Parkin, also account for a significant number of AR-JP cases (Hardy *et al.*, 2009; Narendra *et al.*, 2012; Lazarou *et al.*, 2013). A number of diverse Parkin substrates have been postulated to be associated with PD. There is substantial evidence that one role for Parkin is at mitochondria. Once activated and recruited to damaged/depolarized mitochondria by PINK1, it ubiquitinates exposed mitochondrial proteins leading to both proteasomal degradation and mitophagy (Narendra *et al.*, 2012; Sarraf *et al.*, 2013). Parkin has also been implicated in cell surface signalling and as a tumour suppressor (see references in Wauer and Komander, 2013).

Parkin encodes five structured domains, beginning with an N-terminal ubiquitin-like domain (UblD) and followed by four domains that each bind two zinc (Zn) atoms (Figure 1A). The most N-terminal of the Zn-binding domains is RING0. C-terminal to this is the RBR, consisting of RING1, the IBR and RING2. The crystal structures of inactive Parkin from Riley *et al.* (2013), Trempe *et al.* (2013) and Wauer and Komander (2013) show remarkable congruity. Spatially, the IBR is at the complete opposite end of the molecule from RING2, to which it is connected by a partially unstructured ~37 residue linker. This linker includes a two-turn helix, referred to as the repressor element of Parkin (REP) or tether, which binds and occludes the E2 binding face of RING1. RING1 occupies the central position in these structures, and RING0 separates RING1 from RING2 (Figure 1B and C). The latter contains the residue identified by Wenzel *et al.* (2011), and confirmed by all three groups, to be the catalytic cysteine, C431. A lower resolution structure also includes the UblD and places this domain adjacent to RING1 (Trempe *et al.*, 2013). A second unstructured linker connects the UblD and RING0. UblDs are involved in a number of protein–protein interactions and small angle X-ray scattering confirms that this domain is integral to the core structure of Parkin (Spratt *et al.*, 2013; Trempe *et al.*, 2013). Biophysical characterization of Parkin and HHARI suggests that each is a monomer in solution.

RING1 is the only *bona fide* RING domain. All NMR and crystal structures of IBR domains from Parkin, HHARI and HOIP (PDB ID: 2CT7) are in good agreement. The Parkin and HHARI RING2s are structurally highly homologous and share a common Zn-coordinating arrangement with IBR domains. In contrast to the IBR and RING2, RING0 has a distinct arrangement of Zn-coordinating residues (Beasley *et al.*, 2007; Duda *et al.*, 2013; Riley *et al.*, 2013; Spratt *et al.*, 2013; Trempe *et al.*, 2013; Wauer and Komander, 2013) (see Figure 1F of Trempe *et al.* (2013) for the various Zn coordination arrangements).

All of the Parkin crystal structures represent inactive forms of the E3. This is imposed by the quaternary positioning of the domains, which precludes activity in multiple ways. RING0 plays two obvious roles to maintain Parkin in an inactive state. RING0 shares an interface with RING2 and buries C431, making it unavailable as an ubiquitin acceptor. Moreover, RING0 intervenes between RING1 and RING2, creating an insurmountable separation of >50 Å between the active site Cys of an E2 bound to RING1 and C431 (Figure 1B and C). Thus, RING0 must be displaced for ubiquitin transfer to occur. Accordingly, deletion of RING0 results in a marked increase in Parkin autoubiquitination and in C431 reactivity (Riley *et al.*, 2013; Trempe *et al.*, 2013; Wauer

and Komander, 2013). In HHARI, these two inhibitory functions are fulfilled by the C-terminal Ariadne domain, which similarly interposes between RING1 and RING2 (Duda *et al.*, 2013).

Additional inhibition is provided by the REP, which binds to RING1 at the canonical RING-E2 binding site and prevents E2 binding. This provides at least a partial explanation for the impaired ability of Parkin to bind E2 when compared to HHARI, which lacks this element (Duda *et al.*, 2013). A disease-associated REP mutant (A398T) at the RING1 interface increases autoubiquitination (Wauer and Komander, 2013). The significance of inhibition by REP-RING1 binding was verified by mutating a critical RING1-interacting REP residue (W403A). This increased autoubiquitination and E2 binding (Trempe *et al.*, 2013). Consistent with the requirement for charging C431 with ubiquitin in mitochondrial translocation (Lazarou *et al.*, 2013), Parkin association with depolarized mitochondria is accelerated with this mutation (Trempe *et al.*, 2013). Interestingly, W403 also interacts with the C-terminal Val of Parkin within RING2, and could therefore potentially further stabilize the autoinhibited form of the protein (Riley *et al.*, 2013), consistent with previous observations (Henn *et al.*, 2005).

The quaternary structure of full-length Parkin also suggests that displacement of its N-terminal UblD must occur for full activation (Trempe *et al.*, 2013). The positioning of the UblD adjacent to RING1 indicates that it would provide a steric impediment to E2~Ub binding (Figure 1B and C). Additionally, displacement of the UblD could be important to relieve interactions with the IBR-RING2 linker, which, as suggested in a previous study (Chaugule *et al.*, 2011), might help to maintain Parkin in an inactive state. Finally, the crystal structure of the full-length Parkin indicates that the UblD is not available for interactions with other proteins. This would limit Parkin's range of intermolecular interactions.

RBR E3s have at least two domains critical for sequential ubiquitin transfer and full activity, RING1 and RING2. The RING1 of Parkin, as well as all other RBR E3s, is notable in lacking the basic residue in the second Zn coordinating loop (or its equivalent in U-box proteins), which has recently been implicated in RING-mediated transfer of Ub from E2~Ub (Metzger *et al.*, 2013). This suggests that other factors play compensatory roles in positioning ubiquitin for transfer from E2~Ub to C431. A non-mutually exclusive possibility is that the lack of this basic residue in RING1 limits unwanted attack on the E2~Ub linkage, thereby minimizing the unregulated ubiquitination. Turning to RING2, the area surrounding the active site C431 of Parkin is notable in that it includes a sequence recognizable as a catalytic triad, similar to that in deubiquitinating enzymes. The Cys-His-Glu grouping, found in Parkin and other RBR E3s, contributes to *in vitro* activity (Riley *et al.*, 2013; Wauer and Komander, 2013). Interestingly, however, the Glu was dispensable in a cellular assay (Riley *et al.*, 2013). This triad is conserved in HHARI, where an Asn between the Cys and His residues (found in a number of RBRs but not conserved in Parkin), was found to be important for catalysis (Duda *et al.*, 2013).

The advances made in these studies impart significant information about an important and clinically relevant E3. However, Parkin, as well as HHARI, has been captured in their inactive, unmodified forms. One obvious question is

how does Parkin transition between inactive and active states. PINK1 is implicated in phosphorylating Parkin on its UbLD and potentially other sites, with evidence that phosphorylation contributes to Parkin activation (Narendra *et al*, 2012). How phosphorylation could contribute to protein interactions that might facilitate Parkin activation, potentially including Parkin oligomerization (Lazarou *et al*, 2013), is unknown. Regardless, it is evident that considerable unwinding of its quaternary structure must take place.

While there is much work ahead to understand these processes, one important interface that must be disrupted for activation is that between the REP and RING1. It is intriguing to consider that such interruption might be associated with other alterations in the IBR-RING2 linker, potentially facilitating the movement of the UbLD from RING1 and contributing to activation. Related to activation is the all-important question of how Parkin recognizes and targets specific substrates. While the UbLD represents a potential site of interaction, most purported substrates are not known to have UbLD-interaction domains. Although interactions involving the UbLD could occur indirectly, through bridging molecules, there is also evidence that other regions of Parkin, including the RBR region, might recognize substrates either directly or indirectly (Tsai *et al*, 2003) and that some substrates may be phosphorylated by PINK1 (Narendra *et al*, 2012). Conformational changes induced by substrate

interactions, particularly in the IBR RING2 linker, could, as above, represent an important aspect of activation.

There are over 75 missense mutations of Parkin associated with AR-JP, most of these inactivate the protein, but there are also some that are activating (Wauer and Komander, 2013). Activating mutations presumably result in pathology at least partially as a consequence of increased autoubiquitination and degradation (e.g., A398T). The current studies help to provide a classification of missense mutations into those that affect (i) folding or stability, (ii) catalytic mechanism, and (iii) interactions between domains. Interdomain mutations might inactivate or contribute to constitutive activation leading to autoubiquitination and degradation.

Finally, we know little about how the autosomal recessive and the much more prevalent sporadic forms of PD overlap in their molecular pathology. However, mitochondrial dysfunction is increasingly a common theme. Thus, with the structure of the inactive protein in hand, there is hope that we can begin to consider ways in which domain interactions might be altered in a controlled manner to activate, but not hyperactivate, this critical E3 and lessen the progression of PD.

Conflict of interest

The authors declare that they have no conflict of interest.

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