Review

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Parkin and mitochondrial quality control: toward assembling the puzzle

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Parkin is an E3 ubiquitin ligase associated with autosomal-recessive Parkinsonism. Moreover, parkin inactivation has been found in sporadic Parkinson's disease (PD), suggesting a wider pathogenic impact than initially predicted. Beyond its role in PD, parkin has also been implicated in innate immune responses. Since its discovery, mounting evidence indicates that parkin can mediate degradative as well as nondegradative ubiquitination. Here we review recent insights into the structure of parkin, the mechanism of its E3 ligase activity, and its functional versatility in an attempt to merge controversial aspects into a more comprehensive picture of this multifaceted E3 ubiquitin ligase.

Parkin: a versatile E3 ubiquitin ligase

The gene encoding the E3 ubiquitin ligase parkin (PARK2) was originally identified as a causative gene for familial early-onset Parkinsonism [1]. To date, more than 120 different pathogenic parkin mutations in over 600 affected families have been reported, accounting for most cases of autosomal-recessive Parkinsonism [2,3] (http://www.molgen.ua.ac.be/PDmutDB).

Based on the well-known function of E3 ubiquitin ligases in targeting substrate proteins for proteasomal degradation, it was initially hypothesized that the loss of parkin function leads to the accumulation of toxic substrates that damage dopaminergic neurons, consequently causing Parkinsonism. This concept launched the search for parkin-interacting proteins and led to the identification of substrates that are tagged by parkin for degradation by the proteasome (for a review, see [4]). However, research during the past 15 years has revealed that the wide range of parkin activities is probably not limited to the degradative ubiquitination of single substrates but also includes nondegradative ubiquitination, which is implicated in the regulation of fundamental cellular processes (for reviews, see [5–7]).

Although various cellular functions have been attributed to parkin, such as elimination of damaged mitochondria and prosurvival signaling, controversy continues over which of its functions is responsible for its pathophysiological role in PD. In this review, we describe recent studies that have shaped our current understanding of the mechanisms of parkin. Specifically, we discuss structural studies and the reclassification of E3 ubiquitin ligases that have provided profound insights into parkin function. Furthermore, we review the various neuroprotective roles of parkin in mitochondrial quality control and stress protection and describe the recently discovered link between parkin and innate immunity.

Lessons from parkin structure

Gaining insight into the structure of parkin has been a longstanding challenge, probably due to its high cysteine content (35 of 465 amino acid residues) rendering parkin prone to misfolding and hampering the generation of soluble, natively folded recombinant parkin. Recently, several groups have reported on the structure of N-terminally truncated parkin comprising the really interesting new gene (RING)0–RING-Between-RING (RBR) domain [8–11]; one also included the crystal structure of full-length parkin at a lower resolution [8]. The structures reported are similar and provide valuable insights into how parkin activity is regulated and how pathogenic mutations cause loss of parkin function. Based on this structural data, pathogenic parkin mutations can be classified into at least three groups impacting: intramolecular interactions or interactions with E2, substrate, or adaptor proteins; parkin folding and stability; or catalytic activity. We discuss these three groups below.

Parkin belongs to the RBR class of E3 ubiquitin ligases (for a review, see [12]), which combines features of both RING and HECT E3 ligases to transfer ubiquitin to substrate proteins (Box 1). Parkin possesses a modular structure containing a ubiquitin-like (UBL) domain at the N terminus and a RBR domain at the C terminus (Figure 1A). The RBR domain comprises two different RING domains, RING1 and RING2, separated by an in-between RING (IBR) domain. N terminal to the RBR, an atypical RING domain has been identified, named RING0 or the unique parkin domain (UPD).

Overall, parkin forms a relatively compact structure with multiple domain interfaces resembling a coiled snake [13] (Figure 1B). Two critical regions, the active site cysteine in RING2 (C431) and the E2-binding site in RING1, are occluded, indicating a nonactive, autoinhibited state as suggested previously [14,15]. Autoinhibition is

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Box 1. Parkin: a RING/HECT hybrid E3 ubiquitin ligase

Until recently, two classes of E3 ubiquitin ligases had been defined – RING ligases and HECT ligases – that differ in the mechanism of ubiquitin transfer to the substrate. Whereas RING ligases function as scaffolds to facilitate the direct transfer of ubiquitin from a ubiquitin-charged E2 to the substrate, HECT ligases form a thioester intermediate with ubiquitin via a catalytic cysteine residue. Subsequently, the ubiquitin moiety is passed on to a lysine residue of a target protein generating an isopeptide bond. RBR E3 ligases were initially considered as a subclass of RING E3s, but recent studies revealed that they use a RING/HECT hybrid mechanism. Similarly to RING ligases, they bind an E2 ubiquitin-conjugating enzyme via their RING1 domain. The ubiquitin is then transferred from the E2 to a conserved catalytic cysteine residue in RING2 forming a transient thioester intermediate, reminiscent of HECT E3 ligases (Figure I). This hybrid mechanism has now been demonstrated for the RBR ligases HHARI, HOIP, HOIL-1L, and parkin and seems to be a general mechanism of RBR E3 ligases [18,19,56,70,116]. Interestingly, an IBR–RING2 parkin fragment is able to mediate E2-independent autoubiquitination *in vitro*, suggesting that, at least under some experimental conditions, this fragment can accept ubiquitin from the E1 ubiquitin-activating enzyme [15,70].

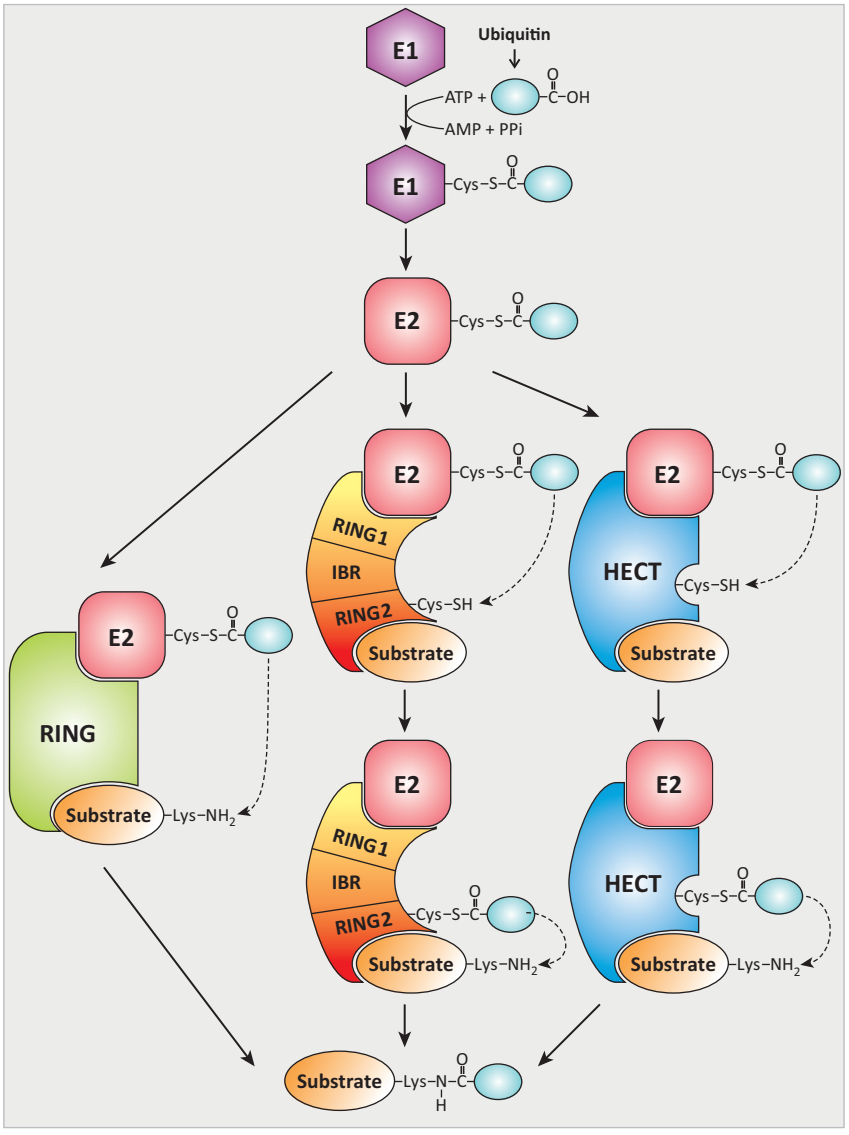

Figure I. Mechanism of ubiquitin transfer mediated by really interesting new gene (RING), HECT, or RING-Between-RING (RBR) E3 ligases. Ubiquitination is accomplished by the coordinated action of three enzymes: an E1 ubiquitin-activating enzyme; an E2 ubiquitin-conjugating enzyme; and an E3 ubiquitin ligase. The E1 uses ATP to activate the carboxyl terminus by adenylation, which allows transfer of ubiquitin to the E1 active-site cysteine forming a thioester. Ubiquitin is then transferred to the E2 active-site cysteine by transthioesterification. The ubiquitin-charged E2 binds to an E3, which mediates transfer of ubiquitin to a substrate protein. RING-type E3 ligases (left) facilitate the direct transfer of ubiquitin from the E2 to a primary amino group of the substrate, usually the ε-amino group of a lysine residue. Similarly to RING ligases, RBR ligases (middle) bind the ubiquitin-loaded E2 via a RING domain. Catalysis by RBR and HECT ligases (right), however, involves formation of an additional ubiquitin–thioester intermediate via an E3 active-site cysteine, from which ubiquitin is transferred onto the substrate to form an isopeptide bond.