Structure of parkin reveals the mechanism of autoinhibition

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Mutations in the gene park2 that codes for a RING-In-Between-RING (RBR) E3 ubiquitin ligase are responsible for an autosomal recessive form of Parkinson’s disease (PD). Compared to other ubiquitin ligases, the parkin protein exhibits low basal activity and requires activation both in vitro and in cells. Parkin is a 465-residue E3 ubiquitin ligase promoting mitophagy of damaged mitochondria. Parkin has two RING motifs RING1 and RING2 linked by a cysteine-rich in-between-RING (IBR) motif, a recently identified zinc-coordinating motif termed RING0, and an N-terminal ubiquitin-like domain (Ubl). It is believed that parkin may function as a RING/HECT hybrid, where ubiquitin is first transferred by the E2 enzyme onto parkin active cysteine and then to the substrate. Here, we report the crystal structure of full-length parkin at low resolution. This structure shows parkin in an auto-inhibited state and provides insight into how it is activated. In the structure RING0 occludes the ubiquitin acceptor site Cys431 in RING2 whereas a novel repressor element of parkin (REP) binds RING1 and blocks its E2-binding site. The ubiquitin-like domain (Ubl) binds adjacent to the REP through the hydrophobic surface centered around Ile44 and regulate parkin activity. Mutagenesis and NMR titrations verified interactions observed in the crystal. We also proposed the putative E2 binding site on RING1 and confirmed it by mutagenesis and NMR titrations. Importantly, mutations that disrupt these inhibitory interactions activate parkin both in vitro and in cells. The structure of the E3-ubiquitin ligase provides insights into how pathological mutations affect the protein integrity. Current work is directed towards obtaining high-resolution structure of full-length parkin in complex with E2 and substrates. The results will lead to new therapeutic strategies for treating and ultimately preventing PD.

Keywords: parkin, E3 ubiquitin ligase, crystal structure