Poster Presentation

MS53.P43

Catalysis of ubiquitin transfer by the RBR ubiquitin ligase parkin

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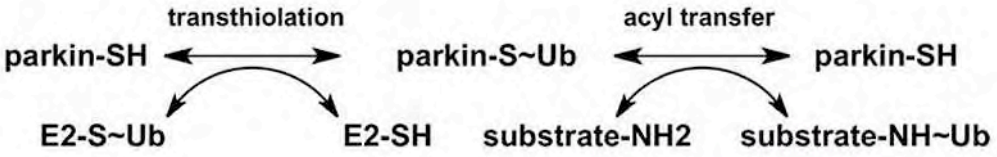
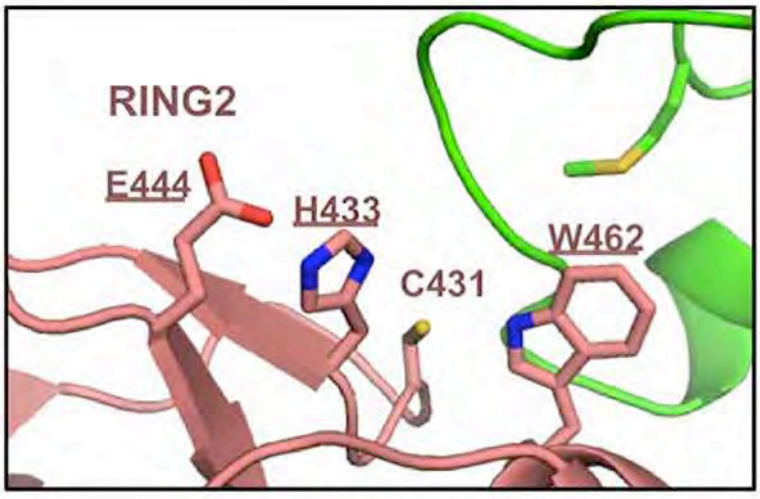
Mutations in the Parkin gene are responsible for an autosomal recessive form of Parkinson's disease. The parkin protein is a RING1-In-Between-RING2 (RBR) E3 ubiquitin ligase, which functions through a two-step mechanism involving a parkin~ubiquitin thioester intermediate [1]. However, compared to other ubiquitin ligases, parkin exhibits low basal activity and requires activation both in vitro and in cells. As parkin is neuroprotective in various models of Parkinson's disease, understanding how it catalyses ubiquitin transfer will be critical. We previously reported the crystal structure of full-length parkin [2]. The structure shows parkin in an auto-inhibited state and provides insight into how it is activated. The RING0 domain occludes the ubiquitin acceptor site Cys431 in RING2 whereas a novel Repressor Element of Parkin (REP) binds RING1 and blocks its E2-binding site. Remarkably, mutations that disrupt these inhibitory interactions activate parkin both in vitro and in cells. The structure also reveals that His433 and Glu444 form a catalytic dyad adjacent to Cys431. Here, we show that His433 catalyses the acyl transfer of ubiquitin carboxy terminus from Cys431 to a target lysine side-chain amino group. Mutation of His433 does not affect UbcH7~ubiquitin discharging or thioester intermediate formation, but prevents formation of polyubiquitin chains on parkin. However, mutation of His433 does not affect significantly parkin's mitochondrial recruitment and substrate ubiquitination, suggesting that other factors might be at play in vivo. We also investigate the catalytic role of other residues located around the Cys431, such as Trp462. The work provides insight into the mechanism of ubiquitination by RBR E3 ligases with important implications for Parkinson's disease.

[1] *D. M. Wenzel, A. Lissounov, P. S. Brzovic, et al., Nature, 2011, 474, 105-108,* ***[2]*** *J.-F. Trempe, V. Sauvé, K. Grenier, et al., Science, 2013, 340, 1451-1455*





Keywords: parkin, ubiquitin, thioester