RESEARCH HIGHLIGHT

Phosphorylated ubiquitin: a new shade of PINK1 in Parkin activation

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The Parkinson's disease (PD)-associated proteins, Parkin and PINK1, together comprise a mitochondrial quality control pathway that promotes neuronal survival through autophagy of damaged mitochondria. Three recent studies have found that Parkin recruitment to mitochondria and ubiquitin ligase activity is controlled by the phosphorylation of ubiquitin by PINK1.

Genetic studies on hereditary early-onset form of Parkinson's disease (PD) have identified disease-causing mutations in a small number of genes, in particular *PINK1* (PTEN induced putative kinase 1) and *PARKIN* (also known as *PARK2*). Since then, functional links between the two proteins have been piling up. In *Drosophila*, mitochondrial dysfunction caused by *Parkin* or *PINK1* mutants can be rescued by *Parkin* over-expression, suggesting that *PINK1* acts upstream of *Parkin* (reviewed in [1]). The PINK1 protein is a serine/threonine kinase that accumulates and becomes active on mitochondria in response to their depolarization, e.g., after CCCP (carbonyl cyanide m-chlorophenyl hydrazone) treatment. The kinase activity of PINK1 is required for the translocation and activation of Parkin, a cytosolic E3 ubiquitin ligase that acts as a quality control guard by ubiquitinating proteins of the outer mitochondrial membrane to trigger selective autophagy of the damaged mitochondria, a process termed mitophagy (Figure 1). Recent structures of Parkin revealed that the protein adopts an inactive conformation under basal conditions and needs to undergo a

structural rearrangement to become active [2–5]. Mutation of residues involved in maintaining the inactive conformation (e.g., a tryptophan in the repressor element of Parkin, or REP) led to an increase in Parkin activity [2].

How does PINK1 recruit and activate Parkin? It has been shown that PINK1 phosphorylates Parkin on serine 65 of its ubiquitin-like (Ubl) domain, leading to an increase of its ubiquitination activity [6]. However, PINK1 must play an additional role in Parkin activation since the non-phosphorylatable S65A mutant of Parkin is still able to translocate to mitochondria (albeit more slowly), whereas PINK1 deletion or mutation completely abolishes translocation [1]. Moreover, the phosphomimetic S65E mutant of Parkin still requires PINK1 for translocation to mitochondria. These observations suggest that there is another substrate for PINK1 in mitophagy.

By using a combination of proteomics screen, Phos-tag-based gel retardation assays and *in vitro* kinase assays, three different groups found that PINK1 directly phosphorylates ubiquitin [7–9]. Mass spectrometry and mutagenesis assays showed that phosphorylation occurs uniquely on serine 65, a residue shared by both ubiquitin and the Parkin Ubl. Thus, upon CCCP treatment, phospho-ubiquitin levels build up on mitochondria in response to PINK1 activation. The phosphorylation of ubiquitin by PINK1 is highly specific: no other ubiquitin-like modifiers were found to be PINK1 substrates and no other kinases tested could phosphorylate ubiquitin.

How does phosphorylated ubiquitin affect Parkin? The same groups demonstrated that phosphorylated ubiquitin stimulates Parkin ubiquitin ligase activity [7–9]. Although the molecular details remain obscure, the effect is independent of ubiquitin's ability to be conjugated. The mechanism of Parkin activation by phospho-ubiquitin appears intimately linked to the Ubl domain. Both Koyano *et al.* [9] and Kazlauskaitė *et al.* [8] found that the phosphorylation of ubiquitin and 'priming' of Parkin, either through deletion or phosphorylation of the Ubl or mutation of the REP, are required for optimal activation of Parkin ubiquitination activity. These observations suggest that the phosphorylation of Parkin on serine 65 exposes a region of the protein that binds phosphorylated ubiquitin. The spatial proximity of the REP to serine 65 in the Ubl of Parkin adds further weight to the functional interplay between phosphorylation and derepression of activity. However, alternative hypotheses exist. Wauer and Komander observed a sulfate anion bound to a basic patch in the Parkin RING0 domain, which is mutated in some cases of PD [3]. As RING0 binds and inhibits the catalytic RING2 domain, binding of phospho-ubiquitin might induce a conformational change to activate Parkin by unblocking the catalytic site.

Ubiquitin phosphorylation activates Parkin ligase activity and also seems to play a role in the translocation of Parkin to depolarized mitochondria. Expression in cells of ubiquitin variants that cannot be phosphorylated leads to a decrease in activation and translocation

Figure 1 Mitochondrial quality control pathway mediated by PINK1 and Parkin. Loss of either protein leads to early-onset forms of Parkinson's disease. Recent reports from three groups have shown that phosphorylated ubiquitin is a key intermediate in the pathway.

of Parkin to damaged mitochondria [7, 9]. Expression of the phosphomimetic ubiquitin mutant S65D did not target Parkin to mitochondria, implying that phospho-ubiquitin needs to be anchored on mitochondria to recruit Parkin. Koyano *et al.* [9] have estimated that 0.05% of total ubiquitin in cells is phosphorylated on serine 65 upon activation of PINK1, thus presumably only ubiquitin in close vicinity of membrane-anchored PINK1 is phosphorylated. The subsequent activation of Parkin and ubiquitination of mitochondrial proteins would provide additional substrates for PINK1, which would then recruit even more Parkin. Such a cycle of amplification would explain why Parkin ubiquitin ligase activity is required for its translocation to mitochondria [10].

The identification of a kinase that phosphorylates ubiquitin is a major breakthrough that links the two most important signaling pathways in cells. Outside of mitochondrial quality control, phospho-ubiquitin may play other roles in regulating Parkin. The behavior of Parkin on mitochondria is strikingly similar to earlier observations of phosphorylation-dependent activation and relocalization of Parkin in neuronal synapses [11]. Parkin can be activated by EGF signaling [12] — conditions which were independently found to generate serine 65 phosphorylation of ubiquitin (www.phosida.com). More generally, the discovery of phospho-ubiquitin as a second messenger heralds a pallet of new colors in signal transduction with the possible existence of

ubiquitin-specific phosphatases, other phosphorylation sites on ubiquitin, and the phosphorylation of ubiquitin-like modifiers, such as SUMO and ISG15.

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