Pink1, the first ubiquitin kinase

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Pink1 and Parkin, identified through studies of hereditary early onset Parkinson’s disease, are involved in mitochondria quality control. Parkin E3 ubiquitin ligase activity is activated by Pink1 kinase activity, although the mechanism is still elusive. Three recent reports uncover a surprising mechanism in which Pink1 directly phosphorylates ubiquitin to boost Parkin activity.

See also: LA Kane et al (April 2014), A Kazlauskaite et al (May 2014) and F Koyano et al (June 2014)

Pink1, a mitochondrial kinase, and Parkin, a cytosolic E3 ubiquitin ligase, function in mitochondrial maintenance. Pink1 is normally imported into mitochondria, cleaved by PARL protease, and degraded by the proteasome. When mitochondria lose their membrane potential, Pink1 starts to accumulate on the mitochondrial outer surface, where it recruits and activates Parkin to trigger protein ubiquitination, eventually leading to mitochondrial dysfunction. Pink1 is normally imported into mitochondria-depolarizing drug (Kane et al, 2014; Kazlauskaite et al, 2014). Surprisingly, a phosphopeptide identified in mitochondria-associated proteins only in wild-type cells derives from ubiquitin, and phosphorylation occurs at the conserved Ser65 residue, which corresponds to Ser65 in the Parkin UBL. Based on structural similarity between ubiquitin and the UBL domain, Koyano et al (2014) hypothesized that Ser65 on ubiquitin may be phosphorylated by Pink1, and indeed ubiquitin in CCCP-treated lysates exhibited a phosphorylation-dependent electrophoresis band-shift, and the same phospho-Ser65 peptide could be identified by mass spectrometry. Quantification showed that phospho-ubiquitin represents 0.05% of total endogenous ubiquitin, although it is not known whether all the pSer65 ubiquitin molecules are associated with mitochondria. The kinase–substrate relationship was further established by in vitro kinase assays, in which recombinant Pink1 could phosphorylate wild-type but not S65A ubiquitin (Kane et al, 2014; Kazlauskaite et al, 2014; Koyano et al, 2014).

Does phospho-S65 ubiquitin play any role in Parkin regulation, and is there any interplay between Ser65 ubiquitin and Parkin UBL domain Ser65 phosphorylation? Using in vitro Parkin E3 activity assays, all three groups provide compelling evidence that both phosphorylations are involved in and required for full Parkin activation. Kazlauskaite et al found that substituting wild-type ubiquitin with S65A ubiquitin greatly diminished Parkin E3 activity, but Parkin with a UBL S65A mutation also had much lower activity compared to wild-type Parkin, indicating that both phosphorylations are required. Kane et al found that prephosphorylation of ubiquitin by recombinant Pink1 could significantly enhance Parkin-mediated protein ubiquitination in vitro. Using GFP-Parkin and purified depolarized mitochondria as a source of Pink1, Koyano et al found that phospho-mimetic S65D ubiquitin, but not WT or S65A ubiquitin, could stimulate autoubiquitination of GFP-S65E-Parkin bearing an additional phospho-mimetic substitution in the UBL domain, but observed no enhancement with GFP-WT-Parkin.

How does the phospho-Ser65 ubiquitin activate Parkin? Koyano et al showed that phospho-mimetic S65D ubiquitin with a C-terminal diglycine mutation, which abolishes its conjugation to target proteins, could still activate the E3 activity of Parkin, excluding the possibility that phospho-S65 ubiquitin is preferred by Parkin for conjugation. They further showed that phospho-mimetic S65D ubiquitin binds to S65E Parkin, but not WT Parkin. The activation mechanism appears to be allosteric and allows functional access of Parkin to its cognate ubiquitin-conjugating E2 enzyme, since phospho-S65 ubiquitin accelerates Parkin discharge of UBCH7–ubiquitin (Kazlauskaite et al, 2014; Koyano et al, 2014). In summary, these studies uncover a surprising missing link in Parkin activation (Fig 1).

To understand how phospho-ubiquitin activates Parkin, the next step is to identify the exact binding site for phospho-Ser65-ubiquitin on Parkin, which was not
UBL domain greatly diminished ubiquitin trapping by Parkin bearing a serine substitu-
tion of its catalytic cysteine 431, also indicat-
ing a positive role for the UBL domain
characterized in the three recent studies.

Interestingly, Wauer and Komander (2013) structurally identified a potential phospho-
peptide binding site in Parkin’s ‘Ring0’ domain, a highly conserved region between the
UBL and the catalytic RBR domains. By
determining crystal structures of Parkin, both Wauer and Komander (2013) and
Trempe et al (2013) found a novel mecha-
nism of Parkin regulation, in which the
Ring0 domain inhibits Parkin E3 activity by
blocking off the catalytic Cys431 in the RBR
domain, such that activation of latent E3
function is ended by dephosphorylation. A mito-
chondrial membrane potential (Sekine et al,
2012). It will be interesting to test if PGAM5
may also affect linkage type or chain elonga-
tion. Presumably, phosphorylation of ubiquitin and
other ubiquitin-like modifiers in general.

Ubiquitin phosphorylation has been docu-
mented on S65, S57, T7, T12, Y59 in previ-
ous phospho-proteomic studies (referenced in
Kane et al, 2014; Kazlauskaite et al, 2014),
although the responsible kinases and
especially their functional importance had
remained elusive. Presumably, phosphoryla-
tion can change the binding properties of
ubiquitin and ubiquitin chains, and phos-
phorylation occurring before conjugation may also affect linkage type or chain elonga-
tion.

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Figure 1. Model of how Pink1-mediated ubiquitin and UBL phosphorylation may activate Parkin.
Pink1 phosphorylates both ubiquitin and the Parkin UBL domain at the Ser65 residue. Parkin UBL domain
phosphorylation exposes a phospho-Ser65 ubiquitin binding site, and phospho-ubiquitin binding in turn
activates Parkin E3 activity.

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Conflict of interest
The authors declare that they have no conflict of interest.

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