SUMO wrestling with Drp1 at mitochondria

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Mitochondria undergo dynamic fission and fusion events, and disruptions in this balance can lead to cellular dysfunction or death. New work by Henley and colleagues identifies a key role for the SUMO protease SENP3 and its target Drp1, a key mediator of mitochondrial fission, in ischaemia.

The term mitochondrion is derived from the Greek *mitos* (thread) and *khondrion* (small grain), emphasizing the fundamental nature of its varied tubular and vesicular appearances within cells. Even this description belies the highly dynamic nature of mitochondria, which undergo continuous cycles of fission and fusion. Maintaining the proper balance between these competing processes is crucial for cellular homeostasis, and a variety of regulatory mechanisms fine tune this balance in different cellular contexts. The key players in mitochondrial fusion in metazoans are large, membrane-bound GTPases in the dynamin superfamily—mitofusin-1 and -2 and optic atrophy-1 (OPA1). Although a number of proteins including Mff, MiD49/51, Fis1, and actin cytoskeletal proteins have been implicated in the regulation of fission, they appear to do so predominantly through their effects on the dynamin-related GTPase Drp1, a protein present mostly in the cytoplasm that is specifically recruited to the mitochondrial outer membrane. After recruitment, Drp1

functions through a complex cycle comprising higher-order oligomerization to form a fission complex, GTPase-dependent constriction that triggers mitochondrial fission, and subsequent Drp1 disassembly and return to the cytoplasm as dimers/tetramers (Otera *et al*, 2013).

Post-translational modifications of Drp1 play important regulatory roles in mitochondrial fission. Known modifications include protein phosphorylation mediated by multiple different kinases, SUMOylation, ubiquitylation, S-nitrosylation, and O-linked-N-acetyl-glucosamine glycosylation. Each Drp1 protomer has four major domains: an N-terminal GTPase domain, a middle assembly domain, a B (or variable) domain, and a C-terminal GTPase-effector domain (GED). Interestingly, the majority of modification sites are found on residues within the variable B domain, with a smaller number in the adjacent GED domain. To add further complexity, there are several known differentially spliced mRNA cassettes within the variable B domain in human Drp1. These modifications can be altered in numerous physiologic and pathologic states, including cell division, neurodegenerative diseases, starvation, and programmed cell death, with often profound effects on the cellular mitochondrial fission/fusion balance (Chang and Blackstone, 2010; Strack and Cribbs, 2012; Otera *et al*, 2013).

Figure 1 Drp1 SUMO-2/3-ylation pathway. During ischaemic stress, SENP3 is degraded, prolonging Drp1 SUMOylation and shifting Drp1 localization to the cytoplasm. Reoxygenation-induced SENP3 recovery results in deSUMOylation of Drp1, with increased Drp1 localization to the mitochondria and subsequent mitochondrial fragmentation, cytochrome c (cyt c) release, and cell death. S, SUMO-2/3 modification.

SUMOylation of substrate proteins has been of particular interest in experimental models of ischaemia, with a number of studies showing global increases in SUMOylation during ischaemic stress, particularly in the brain (Núñez-O'Mara and Berra, 2013). Importantly, SUMOylation induction is protective during cerebral ischaemia, and SUMOylation is increased during hibernation torpor, a model of natural tolerance to ischaemia where cerebral blood flow can fall to 10% of baseline for weeks at a time (Lee *et al*, 2011, 2012). There are three human SUMO paralogs (SUMO1–3), which are conjugated to lysine residues in target proteins via a cascade comprising an E1 complex (SAE1/SAE2), a single E2 (Ubc9), and various E3 ligases. SUMO-2 and -3 are nearly identical in amino acid sequence, and are often referred to and studied collectively; SUMO-1 is only about 45% identical to the others. SUMO proteins are removed from substrates by the SENP family of isopeptidases. There are six SENPs in mammals, SENP1–3 and SENP5–7, with SENP3 and SENP5 favouring removal of SUMO2/3 over SUMO1. The dynamic balance between activities of Ubc9 and SENPs determines the SUMOylation state of proteins.

In this issue of *The EMBO Journal*, Guo *et al* (2013) show that the deSUMOylating enzyme SENP3 is degraded acutely during oxygen/glucose deprivation, an *in vitro* model of ischaemia, through a pathway involving the unfolded protein response kinase PERK and the lysosomal protease cathepsin B. This depletion of SENP3 prolongs SUMOylation of the Drp1 GTPase, driving it into the cytoplasm and suppressing mitochondrial division, cytochrome *c* release, and caspase-mediated cell death. However, upon reoxygenation SENP3 levels rise, deSUMOylating Drp1 and increasing its association with (and division of) mitochondria, cytochrome *c* release, and cell death. Importantly, depletion of SENP3 by RNA interference protected cells from reoxygenation-induced cell death in a manner that required Drp1 SUMOylation (Figure 1). Thus, the Guo *et al* (2013) study provides a potential mechanism for the reperfusion injuries that are so important in the pathogenesis of a variety of disease-related vascular events, including acute cerebral ischaemia. These findings take on added significance, because a recent study of acute spinal cord injury in rodents also found an increase in SENP3 expression peaking several days after the injury, before declining (Wei *et al*, 2012). This latter study also found that inducing oxidative stress with H₂O₂ in pheochromocytoma-derived PC12 cells resulted in increased levels of SENP3 over a period of hours, in step with apoptosis.

Yet, the more general roles of Drp1 SUMOylation in cell death pathways are more complex. McBride and colleagues have previously shown that SUMOylated Drp1 is more stable and has increased residence time on the mitochondrial outer membrane, and also that SUMOylation (mostly SUMO-1) is increased during staurosporine-induced apoptosis in a Bax/

Bak-dependent manner, with an increase in mitochondrial fragmentation. They identified SENP5 as a protease that cleaves SUMOylated mitochondrial substrates, including Drp1 (Wasiak *et al*, 2007). Despite the seemingly opposite effects of SUMOylation on mitochondrial fission between this latter study and that of Guo *et al* (2013), SUMOylation itself seems likely to occur at the same few sites within the B domain of Drp1 (Figuroa-Romero *et al* (2009); Guo *et al*, 2013). This situation is reminiscent of the dual effects of identical protein phosphorylation modifications on Drp1 function. For instance, whereas protein kinase A phosphorylation at Ser 637 (human Drp1 splice variant 1 numbering) is associated with decreased Drp1 activity and inhibition of mitochondrial fission, CaM kinase 1 α - or ROCK1-dependent phosphorylation at the same Ser residue increases mitochondrial fission (reviewed in Chang and Blackstone, 2010; Otera *et al*, 2013).

There are several compelling mechanisms for how the same, or highly similar, Drp1 modifications can give rise to such different functional outcomes. First, modifications could occur at different times and locations during the Drp1 activity cycle, with different impacts. Second, distinct signalling cascades could induce a variety of alterations in other proteins coincidentally. For example, in addition to its role in Drp1 deSUMOylation, SENP3 is essential for rRNA processing, angiogenesis, and cell proliferation, and it translocates from the nucleoli to the nucleoplasm in response to modest increases in reactive oxygen species. Finally, Drp1 itself may have different combinations of post-translational modifications depending on the signalling cascades involved, with cross-talk among them.

It is increasingly apparent that the Drp1 GTPase is the predominant mediator of mitochondria division. A broad range of interacting and modifying proteins provides Drp1 with the flexibility to respond to diverse stimuli, but also exposes a vulnerability to insults. The pathway described by Guo *et al* (2013), in which SENP3 stability acts as a switch to regulate SUMO-2/3-ylation of Drp1 and consequently cytochrome *c* release and apoptosis, presents a potential therapeutic target for increasing resistance to ischaemic stress, with important implications for minimizing damage from acute ischaemia and possibly other injuries.

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Conflict of interest

The authors declare that they have no conflict of interest.

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