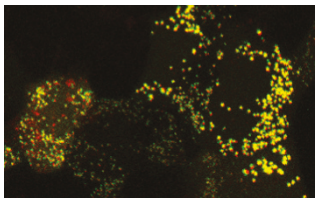
Released from the chains of death



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The mitochondria-associated kinase PINK1 and the cytoplasmic E3 ubiquitin ligase parkin mediate the recognition and disposal of damaged mitochondria by mitophagy; failure of this pathway has been associated with early-onset familial Parkinson's disease. PINK1, degraded upon entry into healthy mitochondria, accumulates on the surface of damaged mitochondria, where it recruits parkin to ubiquitinate substrate proteins. Sensing of the ubiquitinated proteins activates the ubiquitin-proteasome system and triggers an autophagic pathway leading to mitochondrial degradation. However, ubiquitination of mitochondrial surface proteins is kept in check by the surface-bound deubiquitinase USP30. Kirkpatrick, Bingol, Corn and colleagues now explore the mechanistic interplay between USP30 and parkin in the coordination of mitophagy. Induction of mitochondrial damage with the membrane-depolarizing agent CCCP in parkin-overexpressing cells had previously identified mitochondrial parkin substrates. Here the authors analyzed parkin-dependent ubiquitin linkages associated with mitochondrial fractions and found enrichment of Lys63- and atypical Lys6- and Lys11-linked ubiquitin chains. Interestingly, USP30 showed preferential activity toward Lys6-linked ubiquitin *in vitro*. In addition, purified USP30 removed Lys6- and Lys11-linked chains from anchored substrates on intact mitochondria isolated from CCCP-treated cells. Proteome-wide changes in ubiquitination upon USP30 overexpression identified a set of USP30 substrates, several of which overlap with known parkin substrates, suggesting that this subset of proteins may undergo coordinated regulation by the two enzymes. That USP30 activity has a major role in inhibiting ubiquitin-directed organelle degradation was further exemplified by its ability to block pexophagy upon ectopic expression on the surfaces of peroxisomes. These findings provide mechanistic insight into a pathway, governed by the intricate balance between ubiquitination and deubiquitination, that regulates homeostasis of mitochondria and possibly other organelles. (*Nat. Cell. Biol.* **17**, 160–169, 2015) *SG*

m^{6}A drives structural changes in RNA

N^6-methyladenosine (m^{6}A) is the most abundant RNA modification in eukaryotes, present in a large subset of the transcriptome. m^{6}A is thought to act as a dynamic switch that affects processes such as RNA degradation, localization and splicing. Although 'writers', 'erasers' and 'readers' of this mark have been identified, the mode by which m^{6}A exerts its functions is still poorly understood. Parisien, Pan and colleagues now demonstrate that m^{6}A controls RNA-protein interactions by altering local RNA structure. The authors initially identify heterogeneous nuclear ribonucleoprotein C1/C2 (hnRNP C) as a protein that preferentially interacts with an m^{6}A-modified hairpin originating from the long noncoding RNA MALAT1. hnRNP C affects pre-mRNA processing and binds nascent RNA transcripts via single-stranded U tracts. Curiously, the hnRNP C–binding site in the MALAT1 hairpin is located opposite the m^{6}A-modification site. Adenosine methylation is known to destabilize RNA duplexes, and indeed m^{6}A modification of the MALAT1 hairpin increased the accessibility of its opposing U tract. Transcriptome-wide analyses indicated that these 'm^{6}A switches' are common: 7% of all hnRNP C–binding sites corresponded to an m^{6}A-modified consensus motif located in the vicinity of a poly(U) tract. hnRNP C PAR-CLIP experiments in cells expressing siRNAs targeting METTL3 and METTL14, two enzymes involved in m^{6}A deposition, demonstrated that a substantial number of the identified m^{6}A switches showed decreased hnRNP C binding upon m^{6}A depletion. Subsequent analysis of these 'high-confidence' m^{6}A switches supported the finding that m^{6}A influences hnRNP C binding by altering RNA structure, with striking effects on RNA abundance and splicing. The identification of m^{6}A as a structural remodeler opens a new perspective on its mechanism of action, and it will be interesting to learn how many other RNA-binding proteins are affected by this mechanism. (*Nature* doi:10.1038/nature14234, 26 February 2015). *AS*

Written by Cosma Dellisanti, Shubhendu Ghosh & Anke Sparmann

TSPO through the crystal looking glass

The well-conserved transmembrane translocator proteins (TSPOs) were first identified as peripheral benzodiazepine receptors, owing to their affinity for Valium. In eukaryotes, TSPOs have been implicated in transporting cholesterol across the mitochondrial membrane for steroid biosynthesis, although this function has been disputed by recent studies showing that TSPO-knockout mice are not steroid defective. TSPOs also bind to porphyrins, including heme and its precursor PpIX, and a bacterial TSPO was recently shown to catalyze the photo-oxidative enzymatic degradation of PpIX. Despite their presence across all kingdoms of life and their involvement in human pathologies from cancer to cardiovascular diseases and neurological disorders, TSPOs' mechanism and physiological roles remain unclear. Two groups have now independently reported crystal structures of bacterial TSPOs, providing important clues to decipher the confounding functions of these proteins. Hendrickson and colleagues solved structures of *Bacillus cereus* TSPO in monomeric and dimeric forms and provided atomic details of the binding to the benzodiazepine-like drug PK-11195. Ferguson-Miller and colleagues reported structures of *Rhodobacter sphaeroides* TSPO—all showing the protein as dimer—including a porphyrin-bound form and a mutant mimicking a disease-associated polymorphism. All crystal structures showed substantial similarity, with each protomer comprising five transmembrane helices, and were similar to a previous NMR structure of mouse TSPO, which, however, displayed distinct relative positions and orientations of some helices. Ferguson-Miller and colleagues showed that mutations analogous to the human disease–associated A147T polymorphism decrease binding to cholesterol and porphyrin, and Hendrickson and colleagues demonstrated that these mutants are unable to catalyze PpIX degradation. What about function? Interestingly, the dimer interface seen in the crystal structures is too tight for a transport pathway, but Ferguson-Miller and colleagues observed several monoolein molecules bound to protein-surface grooves; this led them to suggest that transport of cholesterol might occur along the external surface of TSPO, possibly with the involvement of other proteins. In addition, biochemical analyses by Hendrickson and colleagues showed that enzymatic degradation of PpIX to a new heme derivative, bilindigin, is a general feature of TSPOs, thus suggesting that the protein might protect cells against oxidative stress. Although many questions remain, these new structures provide a basis to elucidate TSPOs' mechanism and physiological roles. (*Science* **347**, 551–555, 2015 and *Science* **347**, 555–558, 2015) *CD*