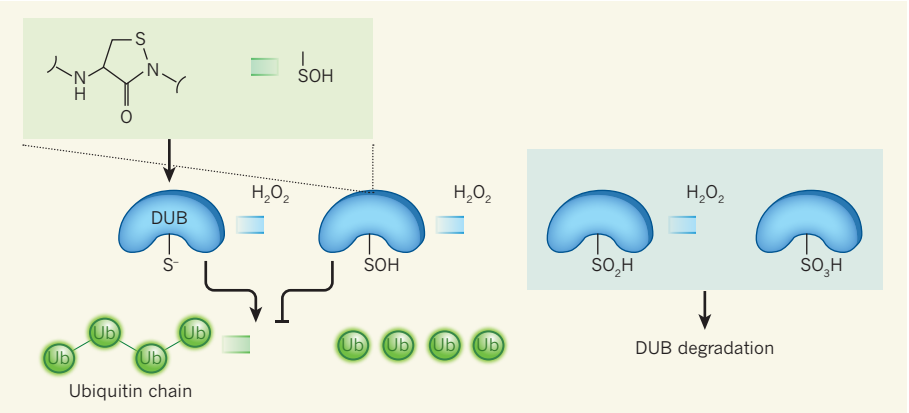

Figure 1 | Redox regulation of deubiquitinating enzymes (DUBs). The active site of many DUBs contains a cysteine amino-acid residue. Deprotonation of this residue results in the creation of a nucleophile (S^-), which allows the enzymes to attack and disassemble ubiquitin chains. However, Kulathu *et al.*[2] and Lee *et al.*[3] show that cysteine deprotonation also renders DUBs vulnerable to oxidation by reactive oxygen species, such as H_2O_2, creating a sulphenic acid group (SOH) at the cysteine residue. This oxidation inhibits the activity of the enzyme, but is reversible in the presence of a reducing agent, as long as further oxidation to sulphinic acid (SO_2H) or sulphonic acid (SO_3H) is avoided. Such oxidation would probably lead to degradation of the enzyme. Lee *et al.* show that one mechanism by which further oxidation is prevented is reaction of the SOH group with a nitrogen atom in a neighbouring residue to generate a reversible sulphenylamide species (green inset).

of enzymes affected by such oxidation have been the tyrosine phosphatases, several of which are directly inhibited by ROS[4]. Now, deubiquitinases are shown to be similarly susceptible.

Ubiquitin is a small polypeptide molecule that covalently attaches to proteins either singly or in the form of polymeric chains. Deubiquitinase enzymes (DUBs) disassemble ubiquitin chains and strip them from their substrate proteins[5]. Through this activity, they can rescue proteins from ubiquitin-dependent degradation pathways or contribute to the dynamics of ubiquitin-mediated signalling. Humans have around 80 DUBs, divided into five subfamilies, of which four comprise cysteine proteases (the USP, UCH, OTU and Josephin subfamilies) and one contains a metalloprotease (the JAMM subfamily). Although the overall architecture of the catalytic domains of the four classes of cysteine-protease DUB are highly divergent, most of the enzymes share a triad of amino-acid residues that adopts a structurally conserved disposition at the enzyme's active site. Crucially, one member of this triad is a histidine residue that deprotonates the active-site cysteine, thereby creating a nucleophilic (electron-donating) site that facilitates attack on the isopeptide bond of the substrate.

Kulathu *et al.* and Lee *et al.* used *in vitro* assays to assess how physiologically relevant concentrations of hydrogen peroxide (H_2O_2) — a source of ROS — affect the activity of purified DUB enzymes. They observed widespread inhibition of the enzymes, but show that this can be readily reversed by an excess of a reducing agent such as dithiothreitol. The authors provide multiple examples of this behaviour from the USP, UCH and OTU subfamilies, and use a combination of mutational analysis, mass spectrometry and structural studies to show that the inhibition is the result of the conversion of the enzymes' active-site cysteine to sulphenic acid (SOH) (Fig. 1).

For this modification to function as a reversible switch, further oxidation to sulphinic and sulphonic acid (SO_2H and SO_3H) must be avoided. The authors present two strategies by which this can be achieved. Lee *et al.* show that, for USP19, the oxidized cysteine bypasses further oxidation by reacting with a nitrogen atom in a neighbouring residue to generate a reversible sulphenylamide species (Fig. 1). Kulathu and colleagues' structural analysis of A20, an OTU enzyme with an SOH group at the active site, suggests that the architecture of the catalytic site provides the opportunity for hydrogen bonding that decreases further oxidative reactivity.

It is likely that many DUBs must continue to function under conditions of oxidative stress. How, then, do they avoid the negative influence of ROS? One suggestion comes from observations that some DUBs crystallize in inactive conformations. In these cases, the distance between the catalytic cysteine and histidine residues is too great for effective deprotonation. It is proposed that binding of the enzyme by its substrate or another molecule is required to realign the enzyme such that these residues come into their active configuration.

The regulation of ubiquitin-dependent signalling pathways by ROS was established[6] in a study which showed that treating cells with H_2O_2 enhances an inflammatory response involving the NF-κB signalling pathway. This coincided with ROS-dependent inhibition of the DUB enzyme Cezanne. Lee and colleagues sought further cellular manifestations of the influence of ROS on DUB-controlled processes. They showed that treating white blood cells called macrophages with H_2O_2, or stimulating them through Toll-like receptors to produce their own H_2O_2, leads to an overall reduction in cellular DUB activity. The authors also find that H_2O_2 treatment leads to the accumulation of the ubiquitinated form of PCNA, a protein that coordinates a DNA-repair pathway for mending damage caused by oxidative stress. Ubiquitination of PCNA, which is required for recruitment of repair enzymes, is held in check by the deubiquitinating activity of USP1. The accumulation of ubiquitinated PCNA suggests that H_2O_2 treatment results in direct inhibition of USP1. This finding mirrors those reported in another paper[7] that also generalizes the findings to other DUBs. Collectively, these papers[2,3,7] highlight the ubiquity of ROS sensitivity across the main cysteine-protease families of the DUBs.

Regulation of DUB activity by oxidation is probably important wherever the action of cysteine-protease DUBs coincides with excess ROS generation. Several DUBs have been implicated in growth-factor signalling pathways, and these must now be considered, along with phosphatases, as potential targets of ROS generation that follows stimulation with growth factors. However, a bone fide example of specific DUB regulation by intracellularly generated ROS awaits description. It is also worth considering that the addition of nitric oxide groups by reactive nitrogen species will similarly modify nucleophilic cysteine residues. Thus, it is possible that a similar means of control operates in physiological processes in which signalling by reactive nitrogen species and ubiquitin dynamics intersect, such as in the regulation of synapses between neurons. ■

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CORRECTION

In the News & Views article 'Solar System: Saturn's ring rain' by Jack Connerney (*Nature* **496,** 178–179; 2013), the unit of wavelength in Figure 2 was incorrectly given as millimetres. The correct unit is micrometres.