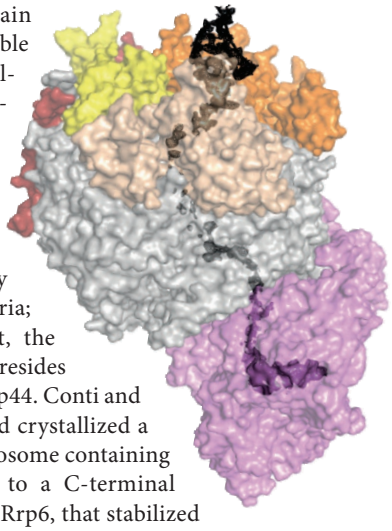
Channelling RNA



The exosome is the main cellular machinery responsible for degrading RNA molecules in the 3′-to-5′ direction and is conserved from archaea and bacteria to eukaryotes. The core consists of a nine-subunit barrel-like structure called Exo-9, which is catalytically active in archaea and bacteria; in eukaryotes, by contrast, the RNase activity of exosomes resides in an additional subunit, Rrp44. Conti and colleagues reconstituted and crystallized a *Saccharomyces cerevisiae* exosome containing Exo-9 and Rrp44, bound to a C-terminal region of another nuclease, Rrp6, that stabilized the complex, and an RNA duplex substrate with a 3′ overhang. The structure reveals the canonical overall architecture of Exo-9, with the C-terminal region of Rrp6 wrapped around the exosome. Rrp44 adopts a closed conformation, with the exoribonuclease region obstructing the exit of the central channel. The first base pair of the RNA duplex is melted just before entering the Exo-9 channel by structural features of the cap proteins Rrp4 and Rrp40—reminiscent of the way that polypeptides are unfolded when entering the proteasome. RNA is then threaded, in a single-stranded conformation, through the narrow entrance pore by sequence-unspecific base-stacking interactions. The channel widens and then narrows again roughly halfway through the barrel-shaped channel and veers sideways, as previously observed in the archaeal exosome. But whereas in the archaeal exosome this sideway channel leads to the phosphorolytic active site of Rrp41, in the yeast exosome the channel ends in the exoribonuclease region of Rrp44, capturing the RNA 3′ end as it exits the side of Exo-9. Thus, although the enzymatic mechanisms differ, the substrate-channelling mechanisms of exosome complexes are conserved from archaea and bacteria to eukaryotes. (*Nature* **495**, 70–75, 2013) *AH*

DUBs redox

Mono- or polyubiquitination events are post-translational modifications that can target proteins for degradation or modify their function; they can be reversed by deubiquitinases, or DUBs, most of which are cysteine proteases. Three recent reports demonstrate that different DUBs are susceptible to regulation by reversible oxidation. In two of the studies, from Ye and colleagues and Komander and colleagues, the authors initially observed that several human DUBs purified from HEK293 cells or bacteria showed low catalytic activity that could be stimulated by the addition of the reducing agent DTT. Those DUBs could also easily, and reversibly, be inactivated by reactive oxygen species (ROS), hinting at the existence of a regulatory redox switch. Komander and colleagues, by using sulfenylation-reactive click chemistry, identified the catalytic Cys103 of the OTU family member A20 as the residue being oxidized. They then obtained high-resolution crystal structures of A20 in various states of oxidation; these structures indicated that Cys-SOH within the catalytic center is a stabilized intermediate protected from further oxidation, which could readily be reduced by DTT. Ye's group, investigating DUBs from the USP and UCH families, also concluded that the catalytic cysteine is the target of oxidation. They went on to demonstrate that DUB inhibition by oxidative stress *in vivo* results in a quantitatively correlated increase in PCNA monoubiquitination, which is a well-characterized response to DNA damage. These observations are in agreement with the findings by Huang and colleagues implicating the oxidation of deubiquitinase USP in maintaining levels of monoubiquitinated PCNA during oxidative stress. Together, these studies indicate that the deubiquitination machinery is responsive to the redox state of cells and might therefore play key roles in various ROS-mediated cellular processes. (*Nat. Commun.* **4**, 1568, doi:10.1038/ncomms2532 and *Nat. Commun.* **4**, 1569, doi:10.1038/ncomms2567, published online 5 March 2013; *Cell Rep.* **2**,1475–1484, 2012) *SL*

PICking H3K4me3

A wealth of information correlates specific histone modifications with gene activity, but the molecular mechanisms by which the former influence transcription-factor function have remained elusive. Now, Roeder and colleagues provide the first insight into how histone H3 Lys4 trimethylation (H3K4me3), a promoter-specific histone modification associated with active transcription, selectively directs the expression of p53 target genes. They show that chromatin templates bearing H3K4me3 support higher levels of p53-dependent transcription *in vitro* than unmodified chromatin, by stimulating the rate of pre-initiation complex (PIC) assembly. Enhanced PIC formation and transcription is mediated by H3K4me3 interactions with the TAF3 subunit of the TFIID complex, as a PHD finger mutation that abolishes TAF3-H3K4me3 binding specifically reduces the stimulatory effect of H3K4me3. Importantly, the presence of H3K4me3 partially suppresses the effect of mutations in the promoter TATA box, suggesting that loss of TATA-box interactions can be compensated by TFIID interactions with chromatin via TAF3. Indeed, the authors show that TAF3-H3K4me3 interactions can act either in concert with the TATA box, to stimulate PIC formation and transcription, or independently, to enhance TFIID recruitment and transcription at promoters containing a mutant TATA box. In cells, depletion of H3K4me3 or disruption of its TAF3 interactions prevents the recruitment of TFIID and RNA polymerase II to p53-regulated cell-cycle promoters but not to proapoptotic p53 targets. Together, these observations establish a direct, gene-specific coactivator function for TAF3 within the p53 network and reveal the mechanism underlying H3K4me3's role in transcription. More generally, they demonstrate how chromatin structure and DNA sequence cooperate to modulate transcription factor activity at both TATA-dependent and TATA-less promoters. (*Cell* **152**, 1021–1036, 2013) *BM*

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