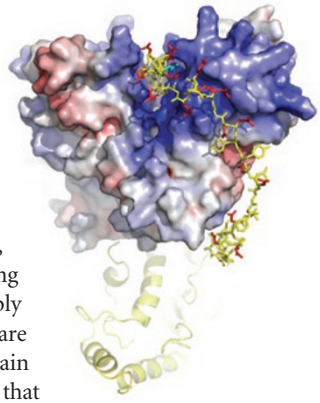
## Pathway to destruction



The addition of four or more ubiquitins to a substrate targets the modified protein to the proteasome, where it is rapidly degraded. Ubiquitin is added through a three-enzyme cascade comprising E1, E2 and E3 enzymes. Although many of the components are well characterized, the mechanism and rates underlying steps in polyubiquitin chain assembly are not clear. In particular, there are two competing ideas for how the chain extends. The stepwise model suggests that each of the ubiquitins is added one by one. Alternatively, the *en bloc* model suggests that the chain is preassembled while still attached to the enzyme machinery and then is transferred fully formed. The sheer speed of these steps has made it almost impossible to distinguish between the models until now. Deshaies and colleagues used a combination of theoretical and experimental techniques to follow ubiquitin transfer to substrate by two different SCF E3 complexes on a millisecond timescale. By analyzing the distribution of ubiquitin tags shortly after initiating a reaction, they found that the first ubiquitin is transferred to the substrate within 10 ms and additional ubiquitins appear in a stepwise fashion. The researchers next addressed the question of how ubiquitin chain assembly can occur so rapidly, given the apparent discrepancy between the high affinity E2-E3 interaction and the rapid cycle of E2 binding to and dissociation from SCF that must occur to enable stepwise assembly of ubiquitin chains on substrate. They found that the E2 that functions with SCF complexes, Cdc34, contains an acidic tail that binds to a basic canyon on Cul1 (the scaffold component of the SCF complex), and this drives Cdc34–SCF interaction with exceptionally rapid dynamics—fast enough to support chain synthesis on a millisecond timescale. Computational analysis suggests that the basic canyon is conserved throughout the cullin family. Together, these studies indicate that a millisecond rate of ubiquitin chain synthesis sustained by electrostatically driven Cdc34-cullin interaction is a conserved feature of all cullin-RING ubiquitin ligases. (*Nature* **462**, 615–619, 2009; *Cell* **139**, 957–968, 2009). *MH*

## The role of YEATS in yeast

In eukaryotic cells, DNA is 'wrapped' around proteins called histones, and these DNA–protein complexes (called nucleosomes) are the building blocks of chromatin. Although many proteins that interact with or modify chromatin are known, it is believed that a large number of proteins involved in these processes have yet to be identified and characterized. *Saccharomyces cerevisiae* Yaf9 is a subunit of the ATP-dependent chromatin remodeling complex SWR1-C (involved in variant histone H2A.Z deposition) and also of the essential histone acetyltransferase complex NuA4 (which acetylates H2A.Z, among other substrates). Yaf9 contains a YEATS domain, a type of domain found in many proteins involved in chromatin biology but whose function was still poorly understood. Wang *et al.* recently determined the three-dimensional structure of the Yaf9 YEATS domain, revealing the presence of a shallow groove with a deep hydrophobic pocket. Because the overall structure was quite similar to that of Asf1, a histone chaperone that binds short peptides, the authors proposed that the groove in the Yaf9 YEATS domain may function as a peptide-binding region. In fact, they showed that Yaf9, like Asf1, could bind to H3 and H4 histones *in vitro*, and mutational studies revealed that evolutionarily conserved residues in the YEATS domain of Yaf9 were required for its function in yeast cells. The authors also determined that the Yaf9 YEATS domain is required for the efficient deposition of histone H2A variant H2A.Z at specific promoters as well as for the acetylation of H2A.Z K14. Interestingly, the putative human ortholog of Yaf9, GAS41, has a YEATS domain that could substitute for its yeast counterpart, supporting the functional conservation of this domain. Future work shall determine whether the shallow hydrophobic groove is the site at which Yaf9 interacts with histone proteins or some other substrate, and also how exactly the YEATS domain participates in histone acetyltransferase and ATP-dependent chromatin remodeling activities (*Proc. Natl. Acad. Sci. USA* doi:10.1073/pnas.0906539106, published online 4 December 2009). *JMF*

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## The short of it

Historically, it was believed that recruitment of RNA polymerase II sufficed to activate transcription. Recent studies, however, have localized RNAP II to inactive promoters, suggesting that transcription might in fact initiate, but then stall early on during elongation. To examine the nature of such stalled transcription complexes, the locations of promoter-proximal RNAP II were mapped with single–base pair resolution throughout the fly genome. Short, capped RNAs (<120 nucleotides) derived from stalled RNAP II were isolated and sequenced. These short RNAs mapped to both active and inactive genes, indicating that transient RNAP II stalling also occurs at genes that undergo productive transcription elongation. These transcripts showed no indication of divergent transcription: most had a single start site at the 5′ end, corresponding to the transcription start site (TSS) of the full-length transcript. These measurements also involved accurate mapping of the transcript 3′ ends, which revealed that polymerase stalling occurred 25–60 nucleotides downstream of the TSS. By calculating the $T_m$ of the DNA-RNA hybrid that would form in early elongation complexes, it was found that genes that did not show stalling had a roughly even $T_m$ profile, whereas genes that did show stalling had a peak in $T_m$ between +20 and +35. Such stalling can be cell type dependent, as genes that showed a peak in $T_m$ but did not display elongation stalling in one cell type produced short RNAs in another cell type. The authors propose that at genes that undergo stalling, RNAP II pauses transiently downstream of the $T_m$ peak and then backtracks to the region of high hybrid stability. This is supported by experiments using cells deficient in TFIIS, a factor that cleaves transcripts in backtracked elongation complexes; in these cells, longer, 35- to 60-nucleotide RNAs accumulated at the expense of the 20- to 35-nucleotide species. Thus, backtracking appears to be an integral part of RNAP II stalling. However, TFIIS-induced transcript cleavage is insufficient to reactivate transcription, and the mechanism by which productive elongation is triggered remains unknown. (*Science* doi:10.1126/science.1181421, published online 10 December 2009). *AKE*