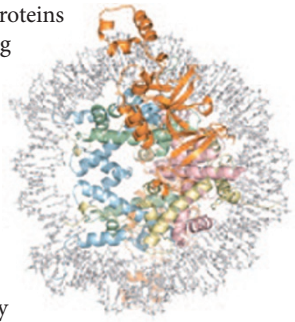
Structural basis of silencing



Silent information regulator (Sir) proteins are essential for silencing of the mating type loci, telomeres and ribosomal DNA loci in budding yeast, and Sir3 can generate silenced nucleosomes *in vitro*. Kingston and co-workers now determine the crystal structure of the bromo-associated homology (BAH) domain of Sir3 in complex with the nucleosome core particle at 3.0 Å resolution. BAH interacts extensively with the histone H4 tail and the regions of H3 and H4 that make up the LRS (loss of ribosomal DNA silencing) domain, which are both known to be important for silencing, as well as H2B adjacent to the LRS surface and the H2A–H2B acidic patch. Importantly, many mutations identified in BAH and in the core histones correlate with residues that form physical contacts between histones and BAH, suggesting that the structure reflects biologically important contacts. Assembly of the complex is accompanied by ordering of the H4 tail and conformational changes in BAH. The structure further explains how covalent modification of H4K16 and H3K79 can modulate silencing. Adjacent nucleosomes in the crystal lattice are bridged by dimerization of BAH, and weak self-association of BAH is also seen in solution. So, although this weak interaction is expected to be insufficient to promote nucleosome compaction by itself, it might contribute to compaction in the context of the full-length Sir3 protein. (*Science* **334**, 977–982, 2011) *AH*

Methylating fingers

To suppress a host cell's immune response, bacterial pathogens such as *Salmonella*, *Shigella* and enteropathogenic *Escherichia coli* (EPEC) target the NF-κB pathway. Recent work in EPEC showed that the type III secreted effector NleE can suppress NF-κB signaling and NF-κB–dependent cytokine production. NleE has orthologs in some species of *Salmonella* and *Shigella*. Shao and colleagues have now determined the mechanism by which NleE inhibits NF-κB signaling. First, they found that NleE can block NF-κB signaling initiated by TNF-α or IL-1β at a step downstream of the TRAF ubiquitin ligases and upstream of TAK1; more specifically, TRAF6-induced TAK1 activation was attenuated. As TRAF6-mediated ubiquitin chain formation was not affected, the authors focused on interactions within the TAK1 complex, comprising the kinase TAK1, TAB1 and the ubiquitin-chain-sensing proteins TAB2/3. TAB2/3 binding to Lys63-linked polyubiquitin chains is required for the oligomerization and autophosphorylation events involved in TAK1 kinase activation. The authors found that NleE targets the Npl4 zinc finger (NZF) domain of TAB2/3, which mediates binding to polyubiquitin chains. Additional experiments revealed that NleE is an *S*-adenosyl-L-methionine methyltransferase that modifies one of the four zinc-coordinating cysteine residues in the TAB2/3 NZF domain. The methylated cysteine is located at a hydrophobic surface that recognizes the Ile44 patch of ubiquitin, so the modification results in loss of ubiquitin binding activity. As zinc binding renders cysteine a better methyl acceptor, and given the prevalence of the zinc-finger motif in many proteins, the authors suggest methylation of a zinc-finger cysteine may play a more widespread but previously unrecognized role in modulating signal transduction. (*Nature* doi:10.1038/nature10690, published online 11 December 2011) *MM*

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Stalled out

Topoisomerase I (TOP1) relaxes DNA supercoiling, permitting the progression of replication forks in proliferating cells. The TOP1 inhibitor camptothecin (CPT) and its derivatives exhibit toxicity toward replicating cancer cells by trapping the TOP1 cleavage complex on DNA, resulting in stalled forks and eventually double-stranded breaks (DSBs). Although the heterodimeric nuclease complex Mus81–Eme1 has been shown to play a role in replication fork rescue and homologous recombination, whether it functions in processing stalled forks due to blocked TOP1 was unclear. Now Pommier and colleagues have shown that Mus81 is involved in alleviating the toxicity of TOP1 inhibition. Cells lacking Mus81 were more sensitive to CPT, but surprisingly, instead of excising inhibited TOP1 from DNA, Mus81 promoted DSB formation at replication foci. The generation of DSBs correlated with Chk2 phosphorylation, indicating activation of the DNA damage response, which would promote repair of the DSBs. These Mus81 effects were specific for replicating cells, and DNA combing of CPT-treated cells showed that Mus81 helps to partially restore the velocity of stalled replication forks. As TOP1 inhibition is known to increase supercoiling of DNA ahead of replication forks, the authors propose that one role of Mus81 is to alleviate the supercoiling, allowing stalled forks to proceed and helping cells to withstand such genotoxic insults. (*J. Cell Biol.* **195**, 739–749, 2011) *SM*

Rli1 does the splits

In bacteria, once translation is completed, the ribosome is recycled into its two subunits using the GTPase activity of recycling factors EF-G and RRF. In eukaryotes and archaea, the mechanism of ribosome recycling is less clear, as some conserved bacterial factors are absent. Instead, yeast uses the conserved ATPase Rli1. Now Shoemaker and Green reconstitute the yeast translation system *in vitro* and define parallel roles for Rli1 in ribosome recycling, with both the classical termination factors eRF1–eRF3 and their paralogs Dom34–Hbs1. Although the Dom34–Hbs1 complex has some ribosome-splitting activity by itself, the presence of Rli1 increases recycling of stalled elongating ribosomes by up to 25-fold. Unexpectedly, GTPase-defective Hbs1 can inhibit Rli1 ATPase activity and ribosome splitting. In fact, Hbs1 GTP hydrolysis promotes its own dissociation from the ribosome, thereby setting the stage for Rli1 action. Subsequent binding of Rli1 stabilizes Dom34 on the ribosome, and both factors actively promote subunit splitting upon ATP hydrolysis. Like in the mammalian system, Rli1–Dom34–Hbs1 can act only on ribosomes that are empty or very near the 3′ end of stalled messages; this length dependence is imposed by Hbs1. In contrast to its mammalian ortholog ABCE1, Rli1 does not require release of the nascent peptide before recycling can occur. Similar principles govern the interaction of Rli1 with eRF1 and eRF3, except that Rli1 also enhances peptide release at stop codons in the context of eRF1, an effect much like that of canonical peptide release factor eRF3, albeit in an energy-independent manner. This work illustrates several mechanistic distinctions between bacterial and eukaryotic ribosomal subunit recycling, and it suggests that eukaryotic recycling is more of a continuum than a highly delineated process. (*Proc. Natl. Acad. Sci.* doi:10.1073/pnas.1113956108, published online 5 December 2011) *AKE*