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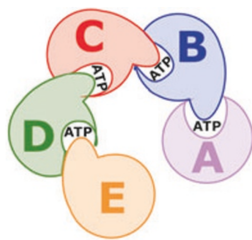
What's in a ring?



Replication factor C (RFC) is a spiral heteropentameric complex that functions in loading PCNA, a processivity factor, onto DNA. As with other multimeric AAA+ proteins, the interface between neighboring subunits constitutes a single ATP-binding site, for a total of four sites in the RFC complex. A recent study from the O'Donnell and Kuriyan laboratories has examined whether each site is equivalent, or whether they perform specific functions in PCNA loading. To do this, the authors mutated residues in the arginine fingers that sense ATP binding and help promote hydrolysis. Various RFC complexes were reconstituted with one mutated subunit and four wild-type subunits or with four mutated subunits and one wild-type subunit. Initially, binding of ATP to the sites helps to torque open the PCNA ring so that it can be loaded onto DNA. ATP bound to the 'C' site specifically facilitates binding of RFC to DNA. ATP hydrolysis in the 'D' site follows interaction with PCNA and promotes closure of the PCNA ring on DNA. This breaks PCNA's association with RFC subunits D and E, and turns off the C-site ATP sensor. Consequently, sequential hydrolysis is activated in the other ATP sites (C to B to A), resulting in dissociation of RFC from DNA so that PCNA can now associate with the replicative DNA polymerase Pol δ. (*J. Biol. Chem.*, published online 15 September 2006, doi:10.1074/jbc.M606090200) *AKE*

Restricting silence

Certain genomic regions, including telomeres, are rich in heterochromatin. However, how heterochromatin is prevented from spreading into neighboring regions where it could silence adjacent genes is not clear. Recent work by Workman and colleagues explores how two chromatin regulatory mechanisms, covalent histone modification and recruitment of variant histones, act together in 'anti-silencing' of subtelomeric loci. This study brings together previous work that independently implicated the histone acetyltransferase complex (SAS) and the histone H2A variant H2A.Z as barriers to heterochromatin spreading. Using microarray analysis in mutant backgrounds, the authors show that expression of telomere proximal genes decreases when the SAS catalytic subunit, Sas2, is deleted. However, the greatest repression was observed when both Sas2 and H2A.Z functions were decreased. SAS seems to act through acetylation of a specific lysine (K16) of histone H4. Further experiments suggest that this activity is required for incorporation of H2A.Z at telomere proximal loci, in a fashion specifically dependent on the presence of both the Sas2 acetyltransferase domain and the histone H4 K16. In contrast, H2A.Z does not influence the abundance of acetylated H4 K16. Altogether, this implies that H2A.Z incorporation occurs downstream of SAS-mediated H4 K16 acetylation. The authors confirmed this by experimentally introducing a SAS recruitment site near a locus with low H2A.Z and acetylated H4 K16 abundance. As predicted, recruitment of SAS, but not of a mutant lacking acetyltransferase activity, to this locus promoted H4 K16 acetylation and H2A.Z recruitment. Further work is needed to establish how SAS targeting to subtelomeric regions is accomplished. (*Genes Dev.* **20**, 2507–2512, 2006) *SL*

Research highlights written by Angela K. Eggleston, Boyana Konforti and Sabbi Lall.

Close up of the ribosome

Two groups have independently solved the crystal structure of the 70S *Thermus thermophilus* ribosome with mRNA and tRNA substrates, allowing a closer look at this complex machine. Observed interactions are consistent with previously described phenomena, such as the third-position wobble, and explain many other biochemical data. The Noller group structure (at 3.7 Å resolution) includes a model mRNA, P (peptidyl) site tRNA and a mixture of tRNA species in the E (exit) site. The authors emphasize conformational changes, discussing shifts in the peptidyl transferase site and a distortion of P- and E-site tRNAs relative to previous structures. Gradual relaxation of such a distortion has been suggested to drive tRNA movement during translation. The Ramakrishnan group describe a 2.8 Å structure, outlining details of ribosomal-tRNA interaction with defined substrates in the A (aminoacyl), P and E sites. tRNA distortion is also observed in this structure, and it may drive translocation to the E site. The authors mapped multiple metal ions, including a Mg^{2+} that stabilizes an mRNA 'kink' between the A and P sites and may prevent mRNA slippage. In addition, they further clarified the nature of the intersubunit bridges (as consisting of protein side chains, RNA and ions), which should be important for understanding changes at the subunit interfaces during translation. These and many other structural insights will drive functional work testing mechanistic models of translation. (*Cell*, published online 7 September 2006, doi:10.1016/j.cell.2006.08.032; *Science*, published online 7 September 2006, doi:10.1126/science.1131127) *SL*

Partners in crime

The p53 tumor-suppressor protein can protect cells from cancer-causing DNA-damaging agents. About half of all human tumors express mutant p53 proteins, and many other cancers involve cellular or viral oncogenes that inactivate normal p53. p53 is organized into three functional domains: an N-terminal activation domain, a central DNA-binding domain and a C-terminal oligomerization domain. Mutated p53 proteins show a decreased ability to bind DNA, altered protein conformations or both, which result in functional consequences that can range from loss-of-function to gain-of-function. Some mutant p53 proteins are highly expressed, which may confer advantages on tumor cells, but how they do so is unknown. Gain-of-function mutant p53 proteins can work by binding, sequestering and inactivating other tumor-suppressor proteins or through transcriptional regulation of target genes. Wild-type p53 regulates the transcription of regulatory genes involved in cell-cycle arrest and apoptosis and also interacts with various transcription factors to regulate genes that lack p53 binding sites. Now Di Agostino *et al.* show that mutant forms of p53 interact with NF-Y, a heterotrimeric transcription factor, and that these mutant p53–NF-Y complexes modulate the expression of key NF-Y–regulated cell-cycle genes upon DNA damage. The transcriptional complex also includes p300, whose acetylase activity might represent the key event that turns on the transcriptional activity of the mutant p53–NF-Y complex, leading to aberrant upregulation of cyclin–CDK1 kinase complexes. These results suggest that the ability of p53 mutants to act as oncogenic factors depends on their ability to interact with transcriptional complexes and activate cell-cycle genes that are normally repressed by wild-type p53, thereby allowing cells to escape cell cycle control and leading to tumor growth. (*Cancer Cell* **10**, 191–202, 2006) *BK*