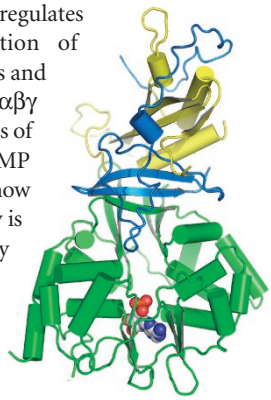
Sensing energy levels



AMP-activated protein kinase (AMPK) regulates cellular metabolism via phosphorylation of metabolic enzymes, transcriptional factors and coactivators. AMPK is a heterotrimeric αβγ complex that can monitor the energy status of the cell: it is activated when bound to AMP and inhibited when bound to ATP. Exactly how these ligands exert their regulatory activity is not yet understood, but a recent report by Townley and Shapiro offers some insights. Upon comparing the crystal structures of a core αβγ complex from fission yeast AMPK, bound either to AMP or ATP, the authors did not observe substantial structural differences outside the nucleotide-binding region in the γ subunit. The α-phosphate of AMP and those of ATP are found within a cavity in AMPK, the 'phosphate tunnel', where they engage the same residues. The smaller AMP molecule is better accommodated than ATP, whose triphosphate group fits by adopting a helical, more compact conformation. Remarkably, AMPK-bound ATP lacks a metal counter-ion, suggesting that ATP must be stripped of its shielding ion for binding to AMPK. These observations may explain why AMPK binds ATP with a lower affinity than AMP. Subtle conformational changes around the phosphate tunnel result in substantial differences in the electrostatic potential at the far end of the tunnel, at a proposed interface with the kinase domain of AMPK. This work begins to show how AMPK detects AMP and ATP levels in the cell and how the adenylate sensor controls AMPK activity. (*Science*, published online 8 February 2007, doi:10.1126/science.1137503) *IC*

A different chain reaction

Lys48-linked polyubiquitination of protein substrates, a mark often used to target proteins for proteasomal degradation, requires an activating enzyme (E1), a conjugating enzyme (E2) and a ubiquitin (Ub) ligase (E3). The addition of multiple Ub moieties to a substrate to form a poly-Ub chain is believed to occur through the sequential addition of Lys48-linked Ub moieties initially added to a substrate lysine. New data from Ye and colleagues now suggest another model to consider in Lys48-linked poly-Ub addition. While examining Lys48 poly-Ub synthesis using the E2 enzyme Ube2g2 and the RING E3 ligase gp78, the authors found that Lys48-linked poly-Ub chains can be assembled on the catalytic cysteine of Ube2g2 when only Ube2g2, gp78 and Ub are present. The authors used a novel two-step ubiquitination assay to show direct transfer of a Lys48-linked poly-Ub from Ube2g2 to a lysine on HERP, an ER-associated protein and a known substrate for gp78. They confirmed poly-Ub chain transfer by Ube2g2 and gp78 using chemically synthesized Ub polymers and demonstrated that poly-Ub chains can be assembled on Ube2g2 and transferred in one step *in vivo*. The authors suggest that gp78 may assist in Ub chain formation on Ube2g2 by promoting dimerization of the Ub-charged E2s. This new 'preassembly' model has some support from previous data on other E2 enzymes: many are able to form Ub chains in the absence of substrate. Further work will be needed to determine the prevalence of preassembly versus sequential assembly of poly-Ub on protein substrates. (*Nature*, advance online publication 18 February 2007, doi:10.1038/nature05542) *MM*

Research Highlights written by Inês Chen, Alexander P. Dorr, Angela K. Eggleston and Michelle Montoya.

Histone merry-go-round

The nucleosome is composed of two copies of each histone (H2A, H2B, H3 and H4) with the DNA wrapped around in a one-and-three-quarters turn. The N terminal tails of histones protrude out of this globular structure and undergo extensive post-translational modification, a key event in the regulation of transcriptional activity. Exchange of nucleosome components during transcription, however, is less well characterized. Imoberdorf and colleagues now describe the transcription-dependent exchange of histone H3 in yeast in the absence of DNA replication. The authors followed the incorporation of histones H2B and H3 into DNA using chromatin immunoprecipitation. They found that H2B occupies the chromatin fiber independent of transcriptional activity, and point mutation of an amino acid residue crucial for interaction with histone H4 disrupts H2B incorporation into the nucleosome. Interestingly, incorporation of both H2B and H3 does not require the N-terminal tail of either. In contrast with H2B, histone H3 exchange is dependent on transcription. Furthermore, the authors show that the previously reported genome-wide decrease in nucleosome density is not permanent but rather reflects a highly dynamic exchange of core histones during transcription. Additional experiments are needed to clarify the potential importance of histone chaperones in this process. (*Mol. Cell* **25**, 345–355, 2007) *APD*

Halt — what goes there?

UV-induced DNA lesions, such as thymine dimers, that occur in the transcribed strand of a gene can be repaired by a process known as transcription-coupled repair (TCR). Biochemical studies have shown that when RNA polymerase II (RNAPII) encounters a lesion, its movement is blocked, with the lesion located in the enzyme's active site. This pausing allows the remaining repair machinery to be recruited for excision of the damaged DNA. Recently, Cramer and colleagues used crystallography to examine how the thymine dimer causes stalling of RNAPII. They solved structures containing both RNAPII and substrates consisting of regions of duplex DNA and RNA-DNA hybrid, with the lesion in various positions. A thymine dimer in the elongation complex was found to enhance the mobility of duplex DNA within the enzyme. When NTPs are added to these substrates, incorporation in the position just upstream of the thymine dimer occurs with normal kinetics, whereas polymerization opposite the two thymines shows slower kinetics. Incorporation of adenine opposite the upstream thymine is retarded because the neighboring template bases cannot twist, owing to the dimer linkage, and thus the polymerase prefers to step backwards. At the downstream thymine, the slower incorporation occurs for a different reason: the enzyme has difficulty in adding any nucleotide, although eventually it can incorporate uracil. Thus, it seems that a transcribed strand lesion first encounters a barrier before it occupies the active site, and the subsequent misincorporation of uracil opposite the 5′ thymine is the rate-limiting step that blocks further translocation, causing the observed RNAPII pause. (*Science* **315**, 859–862, 2007) *AKE*