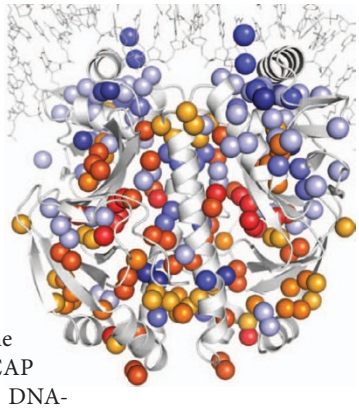
Entropy controls activity



Proteins are inherently dynamic, sampling an ensemble of conformations. How these different conformations contribute to protein activity is not easily quantifiable and is poorly understood. To address this issue, Tzeng and Kalodimos examined DNA binding to several variants of catabolite activator protein (CAP). cAMP binding to the cAMP-binding domain of CAP allosterically switches CAP's DNA-binding domains (DBDs) from inactive states to active states capable of binding DNA. The authors used NMR to examine allosteric mutants of CAP in the absence and presence of cAMP and found that the variants differentially occupied the active DBD state, varying from 2% to 100% occupancy. Unexpectedly, they found no correlation between DNA affinity and increased population of the active state, which suggests that determinants in addition to protein structure played a part in controlling binding affinity. ITC revealed that the variants used different thermodynamic strategies to bind to DNA, with some having favorable entropic contributions and some having favorable enthalpic contributions. Because the DNA-binding surfaces of these mutants were not altered, solvation-energy contributions were similar; however, binding entropies still varied substantially. To examine this further, the authors monitored amplitude changes to fast (picosecond to nanosecond) internal protein motions upon DNA binding. Interestingly, they found that the residues of wild-type CAP-$cAMP_2$ became more rigid when bound to DNA, giving rise to an unfavorable change in conformational entropy. In contrast, residues of two CAP variants that bind with similar affinity as wild type but populate the active state <7% of the time became more flexible upon DNA binding. The authors suggest that this favorable conformational entropic contribution leads to strong binding by these variants and that, in general, the relationship between fast and slow protein motions can regulate protein activity in ways not anticipated from examining a protein's most stable, lowest-energy structure. (*Nature* doi:10.1038/nature11271, published online 11 July 2012) *MM*

The STING of viral fusion

The protein STING (stimulator of interferon genes) has an important role in the mammalian innate immune system. STING was initially thought to function as an adaptor by linking the cytosolic detection of microbial DNA to downstream signaling components and promoting the production of type I interferons. Mice deficient in STING show higher susceptibility to infection by different DNA viruses. More recently, STING was shown to be a sensor for the cyclic dinucleotide c-di-GMP, used by bacteria as secondary messengers. Now, Holm *et al.* find that STING is also involved in the interferon response of cells after viral membrane fusion, in a manner independent of nucleic acids or virus capsid proteins. The authors purified virus-like particles (VLPs) derived from herpes simplex virus I, which lacked capsid or viral genomic DNA but were still able to fuse with cellular membranes. The VLPs could induce an interferon response in both mouse and human cells without causing an inflammatory response. Using VLPs that lacked specific envelope glycoproteins, the authors established that viral membrane fusion is required to induce the interferon response. Other membrane-fusion events, such as cell-cell fusion or cationic liposome fusion, could cause a similar response. Using liposomes with different lipid composition, the authors could see a direct correlation between fusogenic potential and the level of cellular responses. They next investigated the signaling events involved in the cellular response to membrane fusion. Cells from STING-deficient mice did not show interferon responses upon treatment with VLPs or liposomes, implying that protein was needed for the response to membrane fusion. In addition, the roles of phospholipase C-γ and phosphatidylinositol-3-OH kinase, both involved in membrane-proximal signaling, were also demonstrated by using specific inhibitors. The authors note that many membrane-fusion events occur as part of normal physiological processes (for example, ER-membrane fusion or natural syncytium formation) and do not induce immune responses. Thus, cells must be able to differentiate those events from 'unscheduled' membrane fusions that are sensed as danger signals. How exactly membrane fusion is sensed remains to be established, but this work uncovers a novel innate detection mechanism and extends the role of STING in immune responses. (*Nat. Immunol.* doi:10.1038/ni.2350, published online 17 June 2012) *IC*

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Expanding the CTD code

The C-terminal domain (CTD) of RNA polymerase II (Pol II) consists of conserved heptapeptide repeats, which undergo a pattern of phosphorylations during the transcription cycle. Although phosphorylation of Tyr1 of human Pol II was first described almost two decades ago, its functional significance has remained unknown. By developing a phosphorylated Tyr1-specific antibody, Mayer *et al.* were able to demonstrate that transcribing budding yeast Pol II CTD is phosphorylated at Tyr1, in addition to Ser2, Thr4, Ser5 and Ser7, and is correlated with mRNA expression. Tyr1 phosphorylation increases downstream of the transcription start sites of transcribed genes and decreases before the polyadenylation (pA) site. Genomic chromatin-immunoprecipitation profiles of CTD-binding termination factors Rtt103 and Pcf11 peaked downstream of the pA site, whereas that of Nrd1 is consistent with its binding to Ser5-phosphorylated CTD, which suggests that Tyr1 phosphorylation may impair CTD binding of these termination factors. Indeed, none of the termination factors bound to Tyr1-phosphorylated CTD peptide *in vitro*, consistent with this idea. Structural modeling further revealed that Tyr1 phosphorylation causes steric clashes that block the CTD interaction and showed that Thr4 phosphorylation also destabilizes the interaction with CTD-binding termination factors, which is consistent with low Thr4 phosphorylation levels at the pA sites of genes. Finally, the CTD-interacting domain of elongation factor Spt6 binds Ser2-phosphorylated CTD, and given the similar ChIP profiles for Ser2 and Tyr1 phosphorylation, the latter is expected not to interfere with Spt6 binding. Indeed, *in vitro* binding studies showed that Tyr1-phosphorylated CTD peptide stimulates Spt6 binding. So Tyr1-phosphorylated CTD triggers and blocks factor recruitment, thereby expanding the Pol II CTD code. (*Science* **336**, 1723–1725, 2012) *AH*