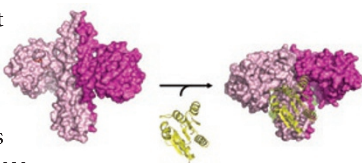
Two-component signaling



Bacteria sense their environment through two proteins, a sensor and an effector. A histidine kinase (HK) acts as the sensor and phosphorylates the response regulator (RR). These highly conserved proteins carry out a four-step reaction, starting with autophosphorylation of HK at a histidine residue. Previous biochemical studies of the HKs EnvZ and NtrB indicated that autophosphorylation takes place in *trans*, with the catalytic domain of one subunit providing the ATP and catalyzing the phosphorylation of the phosphoacceptor histidine, and so it was widely thought that all HKs autophosphorylate in *trans*. When Casino *et al*. solved the structure of the *Thermotoga maritima* class I HK853 and RR468 at 2.8-Å resolution, however, they were surprised to find that in the complex, the β-phosphorus of bound ADPβN is only 11 Å from the His260 εN on the same subunit but is 24 Å from the equivalent atom on the other subunit. This strongly suggested that HK853 autophosphorylates in *cis*. The authors generated point mutations of the key residues, and these confirmed the in *cis* mechanism. In addition, the new structures suggest that extracellular signals are transmitted through the N terminus of HK, which is unstructured in the complex and part of a coiled coil when free. This change appears to derive from a 20° rotation of the coiled coil in the complex that causes the initial three turns to unfold, revealing hydrophobic residues that presumably promote new protein-protein interactions. (*Cell* **139**, 325–336, 2009) *MH*

Initiating Pol II

During transcriptional initiation, RNA polymerase II (Pol II) associates with general transcription factors, and TFIIB (B) is one such key factor. It is required for Pol II recruitment to form the pre-initiation complex, and it contacts both the DNA and the TATA-box binding protein. Since some mutations in B affect transcription start site (TSS) selection and transcription itself, the role of B also extends into post-initiation processes. Cramer and colleagues have now presented a structure of the complete Pol II–B complex at 4.3-Å resolution and from this, along with functional studies and structural modeling, have proposed insights into the processes of initiation and the transition into elongation. The complex is transient, so to facilitate the study, the authors used a linker to fuse B to the Pol II subunit Rpb4. Having solved the structure, the authors find that the B core, consisting of two cyclin domains, binds the wall at the end of the Pol II active center cleft, which is consistent with previous biochemical data and with a role for B in positioning Pol II on promoter DNA. The authors also identify new structural elements in B, including the "B linker" and "B reader." Through mutagenesis studies, they have now shown that the B linker binds the Pol II rudder and clamp and helps to open the DNA. The B reader, meanwhile, sits at the opening of the template tunnel, and functional studies implicate it in TSS selection. In addition, the authors used the previously published structure of TBP complexed to B to model the closed and open promoter complexes. The structure and models are consistent with, and explain, many previous biochemical and genetic insights into the function of B and its role in transcription and provide a framework for future studies into the initiation-elongation transition. (*Nature*, advance online publication, doi:10.1038/nature08548, 9 October 2009) *SL*

Terminate! Terminate!

Upon passing though the protein-coding region of a eukaryotic gene, Pol II continues into the 3′ UTR to the polyadenylation (pA) signal. Although the pA signal is the main determinant of termination, its precise role has been debated. In the torpedo model, cleavage within the transcript's pA signal allows entry of Rat1, a 5′-to-3′ exonuclease that degrades the nascent RNA back to the polymerase, thereby destabilizing template association. In contrast, the allosteric model does not invoke degradation but posits that polymerase processivity decreases after the polymerase passes the pA signal, enhancing Pol II dissociation. Proudfoot and colleagues have now identified two additional modes of termination, after initially finding that termination still occurs in *Rat1*-deficient strains. The first mechanism involves the NRD complex, composed of Nrd1, Nab3 and Sen1, which the authors find binds downstream of the pA site. In a *rat1 sen1* double mutant, readthrough transcription was enhanced. How the NRD complex brings about termination remains unclear, but it likely involves recruitment of the exosome, a nuclease complex. In addition, when a pA signal is absent, a second fail-safe mechanism may ensure termination. This pathway involves an endonuclease, Rnt1, its cleavage sequence (RCS), and Rat1. Here, Rnt1 cleaves the RCS, providing an entry point for Rat1, which then destabilizes polymerase association. Surprisingly, if the RCS is near a weak pA site, it can rescue polyadenylation and stabilize the transcript. This study shows again how sequence context can influence biochemical processes. (*Mol. Cell* **36**, 88–98, 2009) *AKE*

Plant defense

When infected with a bacterial pathogen, plants can trigger so-called hypersensitive cell death at the site of infection, a local response that includes programmed cell death and prevents spreading of the pathogens to healthy tissues. The mechanisms of this process are still mysterious, though a caspase-3–like activity has been reported to function in plant hypersensitive cell death. In animals, caspase-3 plays a key role in apoptosis, with a range of intracellular substrates. Now Hatsugai and colleagues examine the hypersensitive response in *Arabidopsis thaliana* in detail to find that, in response to infection with bacterium *Pseudomonas syringae*, the plant cell large central vacuole fuses to the plasma membrane, releasing the vacuole contents to the outside of the cells. This extracellular fluid can inhibit bacterial growth and also promote cell death, due to the presence of hydrolytic enzymes that degrade the plant cell wall. The authors then examine the requirements for this membrane fusion process, finding that inhibitors of caspase-3 activity or proteasome inhibitors could abolish this effect. In fact, they determined that the plant's caspase-3 activity belongs to PBA1, the β1 catalytic subunit of the plant proteasome. This work thus identifies the plant caspase-3 activity and determines its role in activating membrane fusion to discharge vacuolar content. It will be exciting to see future work revealing the substrates involved in this response and how bacterial infection triggers the process. (*Genes Dev.* published online, doi:10.1101/gad.1825209, 15 October 2009) *IC*

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