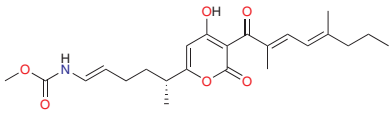
A stochastic switch

The *lac* operon is a model for gene expression control in *Escherichia coli*. In this system, the *lac* repressor (LacI) suppresses transcription of *lac* operon genes by binding to *lac* 'operator' DNA. Lactose or other 'inducers' release LacI from DNA, thereby permitting expression of downstream genes required for lactose metabolism. At intermediate to low inducer concentrations, *E. coli* populations are a mixture of induced and uninduced cells, but the mechanism of the induction process is poorly characterized. A study now suggests that a stochastic repressor-DNA dissociation event may be the molecular trigger that induces this phenotypic switch in individual bacterial cells. Using single-molecule spectroscopy, Choi *et al.* visualized individual YFP-labeled lactose permease (LacY) molecules in living *E. coli* and observed that only cells with several hundred LacY proteins already localized on the membrane underwent induction. The authors surmised that a 'large burst' of LacY synthesis, controlled by the *lac* operon, would be required for cells to reach this induction threshold. Further single-molecule studies of *E. coli* containing engineered operator DNA sequences and LacY surrogates demonstrated that infrequent events involving full dissociation of LacI from the DNA operator produce large bursts of LacY that lead to induction. In contrast, more frequent partial dissociation events of LacI led to incremental LacY production that was incapable of switching cells to the induced state. In addition to providing molecular-level insights into *lac* operon function, the study suggests that stochastic events could more generally control biological phenotypes. (*Science* **322**, 442–446, 2008) *TLS*

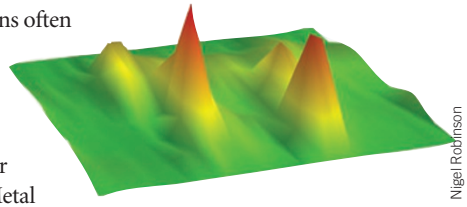
Jamming the switch



Bacterial RNA polymerase (RNAP) provides a suitable target for antibacterial therapy, as it is both essential for bacterial survival and well conserved across bacterial species. RNAP is shaped like a crab claw, and the switch region or hinge located at the base of the claw mediates opening and closing of the clamp. Clamp flexibility is proposed to be necessary to accommodate both initial entrance of template DNA and retention of DNA during transcription. Mukhopadhyay *et al.* use a combination of random and saturation mutagenesis to demonstrate that the antibiotic myxopyronin, a natural product of *Myxococcus fulvus*, inhibits RNAP by interacting directly with the switch region. Biochemical assays strongly support a model in which myxopyronin prevents opening of the RNAP clamp, and thereby precludes entry of double-stranded promoter DNA to the enzyme's active site. Structural analyses confirm that myxopyronin interacts directly with the switch region when the clamp is at least partly closed, which further supports the idea that myxopyronin inhibits transcriptional initiation at the DNA-binding step through an allosteric, hinge-jamming mechanism. Subsequent studies demonstrated that two additional antibiotics, corallopyronin and ripostatin, inhibit RNAP through a comparable mechanism, thus highlighting the switch region of RNAP as an attractive target for future antibiotic drug design. (*Cell* **135**, 295–307, 2008) *AD*

Written by Amy Donner, Catherine Goodman, Joanne Kotz & Terry L. Sheppard

Metallation on location



Nigel Robinson

In vitro metalloproteins often misincorporate metal ions. For instance, divalent metal sites tend to bind Cu^{2+} with higher affinity than Mn^{2+}. Metal selectivity *in vivo* is sometimes controlled by the identity and geometry of ligands in the metal binding site, or through selective metal insertion by metallochaperones. However, in many cases the mechanism for ensuring correct protein metallation is not clear. Tottey *et al.* identified the predominant Cu^{2+}- and Mn^{2+}-containing protein in the periplasm of a cyanobacterium. Structural and spectroscopic data revealed that these two proteins, CucA and MncA, bound their respective metal ions with identical ligands displayed by the same protein fold. As expected, in solution both proteins bound Cu^{2+} selectively over Mn^{2+}. Although once bound Mn^{2+} did not exchange with Cu^{2+}, a four-order-of-magnitude molar excess was required for MncA to bind Mn^{2+} in the presence of Cu^{2+}. In seeking to explain the *in vivo* metal selectivity, the authors noticed that the two proteins are trafficked to the periplasm through different pathways. MncA is exported through the Tat pathway and as a result folds in the cytosol—a cellular compartment with vanishingly little free Cu^{2+} but relatively abundant Mn^{2+}. In contrast, CucA is exported by the Sec pathway and folds in the periplasm, where metal ion concentrations are not as tightly controlled. Although the prevalence has yet to be determined, these results establish folding location as a new mechanism by which cells regulate metal acquisition. (*Nature* **455**, 1138–1142, 2008) *JK*

Navigating through metabolism

Metabolism can be depicted as a complex network of proteins acting on and being regulated by small molecules. Though much of the physical connectivity of this network is known, the ways in which traffic is directed are not as well established. Chechik *et al.* now introduce activity motifs as a way to track dynamic flow through and regulation of known metabolic pathways. The authors first define several 'activation' and 'shutoff' patterns according to a comparison of the timing of the transcription of a gene with the placement of the gene within the relevant pathway. Analysis of expression profiles from time course experiments in yeast using these patterns indicated that the same pathway can be regulated in different ways according to the needs of the cell. For example, addition of menadione, which causes oxidative stress, resulted in a backward-shutoff motif of glycerol production, meaning that production of glycerol, which also increases oxidative stress, was immediately decreased. In contrast, reducing the temperature resulted in a forward-shutoff pattern, or a more gradual decrease in glycerol concentrations, thereby facilitating protein refolding. One underlying mechanism of these directional strategies appears to come from the binding affinity of a common transcription factor for the promoters of the genes in a given pathway, with a higher binding affinity correlating with earlier upregulation and vice versa. This approach to understanding cellular net provides a ready means to test other metabolic hypotheses as well as a conceptual bridge to quantifying cellular behavior and coordination more generally. (*Nat. Biotechnol.*, published online 26 October 2008, doi:10.1038/nbt.1499) *CG*