

HORMONES

Molting with DHR4

PLoS Biol. 9, e1001160 (2011)

Three minor ecdysone pulses in the third-instar (L3) larval stage of *Drosophila melanogaster* development are required to prepare the animal for metamorphosis. It has been difficult to track these pulses because intermediate steps in the ecdysone biosynthetic pathway are not fully understood. To better understand this process, Ou *et al.* demonstrated that decreased expression of the nuclear receptor *Drosophila* Hormone Receptor 4 (DHR4) resulted in precocious ecdysone pulses and elevated concentrations of ecdysteroids during the L3, indicating that DHR4 negatively regulates ecdysone pulses. The authors also noted that the localization of DHR4 oscillated between the nucleus and cytoplasm of ecdysone-producing cells and that nuclear localization of DHR4 was blocked by the activation of a neuropeptide hormone signaling pathway that controls the timing of major ecdysone peaks. Subsequent experiments revealed that the cytochrome P450 gene *Cyt6t3* is a likely target of DHR4: *Cyp6t3* transcript levels oscillated in the L3 larvae, and knockdown of *Cyt6t3* by RNA interference resulted in phenotypes consistent with low ecdysone that could be rescued by feeding larvae 20-hydroxyecdysone (20E), ecdysone or 5 β -ketodiol. Although *Cyt6t3* overexpression alone was not sufficient to accelerate development of wild-type larvae, *Cyt6t3* expression was necessary for the accelerated development of larvae with reduced DHR4. Taken together, these data indicate that *Cyt6t3* encodes one of several enzymes that function in the intermediate steps of the 20E biosynthesis and that DHR4 directly represses *Cyt6t3* expression to help terminate ecdysone pulses.

AD

CELL DIVISION

Pulling apart peptidoglycan

Proc. Natl. Acad. Sci. USA, published online 17 October 2011, doi:10.1073/pnas.1108323108 and doi:10.1073/pnas.1107780108

The ABC transporter-like FtsEX complex is a known component of the cell division apparatus, but its specific function has been unclear. During division, peptidoglycan remodeling is required to separate the two daughter cells: in *Escherichia coli*, EnvC activates the appropriate peptidoglycan hydrolases, but the regulatory mechanisms controlling EnvC and thus limiting undesired hydrolysis are unknown. In *Streptococcus pneumoniae*, PcsB contains hydrolase motifs, and its depletion is associated with defects in cell growth and separation, but biochemical assays have been unable to detect enzyme activity. Yang *et al.* and Sham *et al.* now unify these observations and provide a link between major cell division processes by demonstrating a physical and functional connection between the FtsEX complex and EnvC or PcsB. The two groups used a combination of deletion strains, subcellular fractionation and localization experiments, chemical crosslinking, and mutant constructs to show that the interaction involves an extracellular loop on FtsEX and the coiled-coil domains of EnvC or PcsB. Additionally, the interaction itself dictates both localization and activation of EnvC or PcsB, whereas activation, but not localization, requires ATP hydrolysis by FtsEX for EnvC and probably for PcsB. These results lead to a model in which conformational changes in FtsEX in

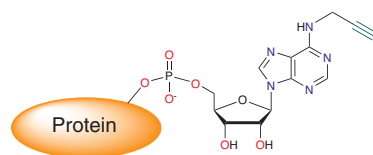
response to ATP hydrolysis propagate to structural changes activating EnvC or PcsB, though the nature of these rearrangements requires further study.

CG

CHEMICAL TOOLS

AMPing up click reactions

J. Am. Chem. Soc., published online 23 September 2011, doi:10.1021/ja205137d



AMPylation is a recently discovered post-translational modification (PTM) reaction that involves transfer of an adenylyl 5'-monophosphate (AMP) group to the side chain of hydroxylated amino acids in proteins. The PTM is installed by AMPylator enzymes that use ATP as an AMP donor and contain either a filamentation induced by cAMP (fic) or adenylyl transferase (AT) catalytic domain. Many AMPylators, such as VopS, are bacterial virulence factors that appear to target eukaryotic small GTPases such as Cdc42, but AMPylators have recently been identified in humans (HYPE) and in *Drosophila melanogaster* (dFic). Though AMPylation appears to be widespread, the role of these PTMs and the substrate scope of AMPylators remains unclear. Grammel *et al.* now report a method for the selective tagging of AMPylated proteins using Cu(I)-catalyzed alkyne-azide cycloaddition reactions, or 'click' chemistry.

The authors synthesized N⁶-propargyl adenosine 5'-triphosphate (N⁶pATP) and demonstrated that it functions as an effective ATP analog for all known classes of AMPylators and that the resulting AMPylated proteins can be selectively tagged with fluorescent or biotinylated groups through click chemistry. This bioorthogonal labeling strategy was applied to the validation of known AMPylation substrates from mammalian cell lysates on a proteome level, suggesting that it may be useful for future investigations of AMPylator substrate specificity in cells. *TLS*

SINGLE-CELL ANALYSIS

Bacteria tuned to FM

Science, published online 6 October 2011, doi:10.1126/science.1208144



In prokaryotes, alternative sigma factors such as the *Bacillus subtilis* σ^B regulate RNA polymerase to activate target genes in response to diverse stresses. In a quantitative time-lapse microscopy study in individual cells, Locke *et al.* now show that energy stress induces a sustained series of stochastic pulses of σ^B activation, with the level of energy stress predominantly regulating the frequency of these pulses. The σ^B pulses arise through amplification of a noise-dependent process. By varying the levels of regulatory components coexpressed in the same operon as σ^B —the RsbS (regulator of sigma B), including the kinase RsbW and the phosphatase RsbQP—the authors showed how pulses originate from a sensitive phosphoswitch that activates σ^B in response to fluctuations in the phosphatase/kinase ratio. Autoregulation results in a mixed positive-negative feedback loop, allowing for both amplification and termination of pulses. RsbQP expression is at the center of this mechanism: high levels engage the positive feedback loop to activate more σ^B , leading to higher induction of RsbW that ultimately turns the system off, leading to a pulse. The results show that bacteria implement highly dynamic, noise-dependent pulsatile response programs to dynamically balance the benefits and costs of σ^B activation.

MB

Written by Mirella Bucci, Amy Donner, Catherine Goodman & Terry L. Sheppard