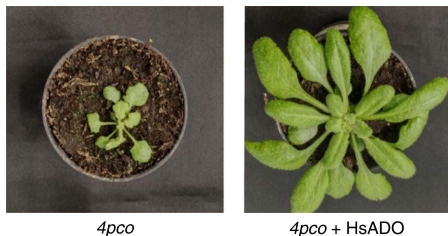
HYPOXIC REGULATION

Breathing the same air

Science **365**, 65–69 (2019)



Credit: Francesco Licausi and Beatrice Giuntoli

In plants, the ethylene response factor transcription factors (specifically, ERF VII TFs, which all have a Met-Cys N-terminal sequence) undergo degradation under aerobic conditions through a ubiquitin-dependent N-degron pathway. Following removal of the initiator methionine by methionine aminopeptidase, the plant cysteine oxidase (PCO) enzymes oxidize the N-terminal cysteine to cysteine sulphinic acid to promote degradation. Masson et al. found that plant and human cells utilize the same N-degron mechanism during hypoxia. They tested two thiol dioxygenases that are structurally similar to PCOs as potential human candidates. Overexpression of cysteamine (2-aminoethanethiol) dioxygenase (ADO) was sufficient to decrease the expression of a known human N-end rule (Cys) substrate, RGS4. Expression of human ADO in quadruple *pco* mutant plants was able to rescue the developmental and hypoxic gene-regulation defects, confirming the shared mechanism. Mass spectrometric analysis revealed ADO-mediated dioxygenation on the N-terminal cysteine of RGS4, which was blocked under hypoxic conditions. This modification promoted high turnover of RGS4 in an oxygen-dependent manner. Finally, Masson et al. identified that interleukin-32 also underwent dioxygenation by ADO, revealing a diversity of potential substrates regulated by this conserved hypoxic degradation mechanism. *GM*

https://doi.org/10.1038/s41589-019-0359-6

AUTOPHAGY

Stick it to proteins

J. Biol. Chem. https://doi.org/10.1074/jbc.AC119.009977 (2019)

LC3 is a small ubiquitous soluble protein important in autophagy, a process of bulk degradation of cytoplasmic components. The cytosolic form of LC3 can be conjugated to membrane phospholipids and recruited to autophagosomal membranes. Given the similarities to the protein-modifying ubiquitin system, including structural homology between LC3 and ubiquitin and a role for deubiquitinase-like cysteine protease ATG4 in proteolytic processing and delipidation of LC3, Agrotis et al. searched for other non-phospholipid substrates for LC3 conjugation. The authors used a deconjugation-resistant form of LC3 and ATG4 knockouts to find that LC3 could stably modify cellular proteins, including ATG3, an E2-like conjugating enzyme involved in the LC3 lipidation reaction. The LC3B–ATG3 conjugates, of which at least one is mediated through ATG3 residue K243, are distinct from the thioester-linked covalent intermediate between LC3B and ATG3 that is known to form before LC3 lipidation. Finally, the authors showed that ATG4B can cleave the LC3B–ATG3 conjugates in a process that is analogous to the delipidation of LC3. These results broaden the scope of LC3 targets and potentially its range of functions. *MB*

https://doi.org/10.1038/s41589-019-0361-z

PHOSPHORYLATION

Switching on germination

Proc. Natl Acad. Sci. USA **116**, 14228–14237 (2019)

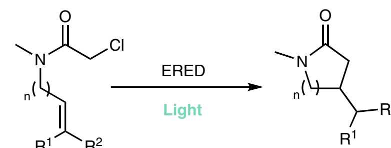
Bacterial spores are highly durable and resilient cells that are dormant during periods of stress. Nutrient binding to cognate receptors in the spore membrane induces germination, restarting cellular activity. Seeking to understand the signaling pathways that are triggered during germination of *Bacillus subtilis*, Zhou et al. profiled Arg phosphorylation, which is mediated by the kinase McsB and the phosphatase YwlE. Deletion of *mcsB* or *ywlE* had an opposite effect, increasing or decreasing germination efficiency, respectively, indicating the importance of Arg dephosphorylation during this process. Phosphoproteomic analysis identified 18 phospho-Arg sites enriched in spores lacking YwlE, including the translational factor Tig and the housekeeping σ factor SigA. An R45D variant of Tig that mimics constitutive phosphorylation stalls germination, impairs protein synthesis, and disrupts Tig association with the ribosome. Analogously, an R365D variant of SigA prevents restoration of transcription, likely by interfering with DNA binding. Although phosphorylation of Tig and SigA alone are not sufficient to halt germination, their effects, along with those of *ywlE* deletion, point to a central role for YwlE in signaling during this process. *CD*

https://doi.org/10.1038/s41589-019-0358-7

BIOCATALYSIS

Lights, enzyme, action!

Science **364**, 1166–1169 (2019)



During chemical synthesis, radical reactions can be facilitated by irradiation with light (photoexcitation). Biegasiewicz et al. have now found that this effect can also be extended to enzymes, demonstrating that irradiation with visible light enables certain flavin-dependent ene-reductases (EREDs) to cyclize α-chloroacetamides into γ-lactams. EREDs generally have large, substrate-promiscuous active sites, allowing them to accept a range of substrates while still providing a suitable scaffold to ensure stereoselectivity. Kinetic and spectroscopic data suggest that a complex between the substrate and the flavin hydroquinone in the enzyme active site promotes the initial electron-transfer step. Variation of the substrates and ERED homologs, occasionally with minimal mutagenesis, also affords different ring sizes and cyclization modes, some of which are difficult to access through small-molecule synthetic approaches. This method of using photoexcitation to promote alternative radical reactions in EREDs is likely also applicable to other classes of oxidoreductases and expands the toolbox of available biocatalytic reactions for forming new carbon–carbon bonds. *CD*

https://doi.org/10.1038/s41589-019-0360-0

Mirella Bucci, Caitlin Deane and Grant Miura