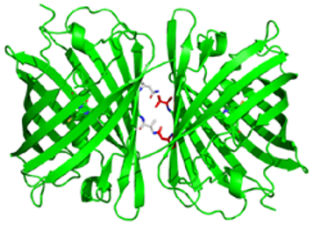
OPTOGENETICS

Lighting up kinases

Science **355**, 836–842 (2017)



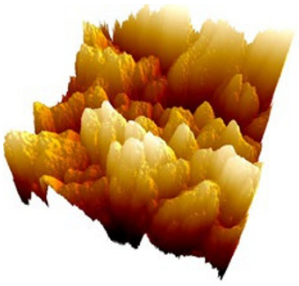
AAAS

The Raf–MEK–ERK kinase pathway, which mediates cellular responses to a variety of extracellular signals, is regulated by positive and negative feedback mechanisms ranging from receptor degradation to transcriptional induction of inhibitory phosphatases. However, detecting these effects with temporal precision remains difficult. Zhou *et al.* engineered photodissociable dimeric variants of the Dronpa fluorescent protein (pdDronpa) that monomerize with cyan-light (500 nm) exposure and then attached pdDronpa domains to two locations on the Raf1 kinase domain. The interaction of the two pdDronpa domains under basal conditions prevents substrate access, while cyan-light exposure promotes dissociation, enabling kinase activity. This effect was reversible such that pdDronpa dimerization was restored with violet light (400 nm), inactivating kinase activity. Zhou *et al.* characterized a fast negative feedback mechanism on MEK, showing that a pulse of MEK activation by pdDronpa-modified Raf1 was followed by dephosphorylation attributable to ERK-mediated activation of protein phosphatases. Finally, the application of this technology to other kinases such as CDK5 provides additional support that pdDronpa may be a generally useful reagent for spatiotemporal control of kinase signaling. *GM*

BACTERIAL MEMBRANES

Tear down this wall

Nat. Microbiol. **2**, 17028 (2017)



SHAWN FRENCH

Infections of Gram-negative bacteria can be particularly difficult to treat, as the bacteria's outer membranes make them impervious to the antibiotics that would kill their Gram-positive counterparts. To identify compounds that could overcome this innate resistance, Stokes *et al.* screened a collection of previously approved small-molecule drugs for their ability to perturb the outer membrane of *Escherichia coli* in a nonlethal manner. The results of this screen identified pentamidine, an antiprotozoal drug that induced rippling in the *E. coli* outer membrane visible by atomic force microscopy, through an interaction with lipopolysaccharide (LPS). Co-treatment with pentamidine made *E. coli* susceptible to rifampicin, novobiocin, and erythromycin, which are normally effective only against Gram-positive strains. Pentamidine also synergized with rifampicin against a variety of Gram-negative species, including strains of *E. coli* and *Klebsiella pneumoniae* harboring *mcr-1*, which confers resistance against last-line antibiotics such as colistin. In a mouse model, co-treatment with pentamidine and novobiocin cleared infections of *Acinetobacter baumanii*, including nearly all cases exhibiting colistin resistance. The use of pentamidine or other similar compounds could give new life to antibacterial drugs facing strains exhibiting either innate or acquired resistance. Furthermore, the fact that pentamidine is already approved for use in humans could smooth its path toward approval for additional uses. *CD*

INTRINSIC DISORDER

Fuzzy fast feedback

Nature **543**, 447–451 (2017)

Recovery from stress due to heterogeneous or fluctuating oxygen levels involves the transcription factor HIF-1α, which is stabilized during hypoxia. HIF-1α and the negative feedback regulator CITED2, one of its downstream targets, both bind to the TAZ1 domain of the transcriptional coactivators CBP and p300 via α-helices and conserved LPXL motifs that exist within intrinsically disordered regions of CITED2 and HIF-1α. These similar binding events raise the question of how CITED2 regulates HIF-1α removal from TAZ1, which is known to be critical for the CITED2 feedback mechanism during hypoxia. To study this, Berlow *et al.* used NMR spectroscopy to characterize what they expected to be a 1:1:1 complex between TAZ1, HIF-1α and CITED2, given the similar binding affinities of the two TAZ1 binders. However, they detected only the TAZ1–CITED2 complex, indicating that CITED2 could fully displace HIF-1α, a conclusion that was supported by fluorescence anisotropy competition experiments. Stopped-flow fluorescence experiments and NMR titrations found a transient ternary complex between HIF-1α, TAZ1 and CITED2. Upon formation of the ternary complex, CITED2 promotes a conformational change in TAZ1 that facilitates HIF-1α dissociation. These findings highlight the functional relevance of intrinsically disordered protein regions in a key feedback mechanism during hypoxia. *MB*

ANTI-BACTERIALS

Out-SMARting drug resistance

Science **355**, 1206–1211 (2017)

Combating antibiotic resistance is crucial in preventing the spread of drug-resistant tuberculosis. Ethionamide (ETH) is a prodrug widely used to treat *Mycobacterium tuberculosis* (Mtb) that is converted to its active form by the bacterial monooxygenase EthA. The transcriptional repressor EthR blocks *ethA* transcription and ETH activation. Following up on their previous work identifying EthR inhibitors, Blondiaux *et al.* characterized a family of spiroisoxazoline-based drugs called Small Molecules Aborting Resistance (SMARt) that failed to bind EthR yet boosted ETH conversion in Mtb, suggesting an alternative pathway for ETH activation. SMARt-420, a representative member of these spiroisoxazolines, upregulated expression of EthA- and EthR-related genes in Mtb (EthA2 and EthR2). SMARt-420 bound to EthR2, preventing its binding to DNA, resulting in induction of EthA2 and increased ETH activation. Importantly, clinical isolates of Mtb with mutations in *ethA* that conferred resistance to ETH were rendered sensitive to the prodrug in the presence of SMARt-420 both *in vitro* and *in vivo*. The findings suggest that identifying alternative pathways of prodrug activation may extend the utility of antibiotics, even in the face of resistance-conferring mutations. *AF*

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