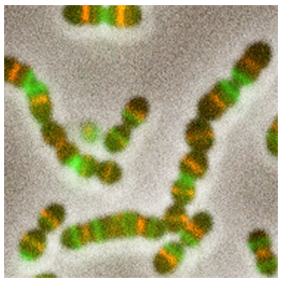
MICROBIOLOGY

TA gets a PG rating

ACS Chem. Biol. https://doi.org/10.1021/acschembio.8b00559 (2018)



Credit: Claire Durmont

Gram-positive bacteria such as *Streptococcus pneumoniae* are surrounded by a cell wall containing a thick peptidoglycan (PG) layer that is covalently modified with linear polysaccharides such as teichoic acids (TA). Targeting cell wall assembly is a common approach for treating Gram-positive infections, though the ability to interfere specifically with TA metabolism is in a nascent stage. Efforts to do so require better knowledge and, therefore, probes capable of monitoring TA metabolism in the context of PG assembly. Unlike TA assembly, PG assembly can be monitored by incorporating fluorescent amino acids into the growing PG. To monitor TA assembly, Bonnet et al. added an azido group to choline, which they found could uniformly label the *S. pneumoniae* surface when a 'clickable' fluorescent probe was introduced to the cells along with the azido-choline. Their two-step, one-pot approach enabled pulse-chase experiments, simultaneously monitoring PG and TA assembly, as well as the determination of localization and timing of TA insertion with respect to the cell cycle and PG assembly. On the basis of these experiments, the authors concluded that TA and PG assembly are largely coincident but TA incorporation persists at the division site later than PG assembly. *MB*

https://doi.org/10.1038/s41589-018-0126-0

SYNTHETIC BIOLOGY

Stuck on you

Science **361**, 156–162 (2018)

In multicellular organisms, signaling networks between cells drive patterns of gene expression that result in morphological changes and organization into complex structures. Using a synthetic Notch (synNotch) receptor system, in which the expression of target genes is driven by ligand–receptor recognition between neighboring cells, Toda et al. engineered cells that self-organize into desired patterns. In their system, different populations of cells express a synNotch ligand or its cognate receptor, with adhesion molecules (cadherins) expressed under control of the synNotch-responsive promoter. When co-cultured, the cells reproducibly self-organize into layered structures, with the arrangement of the various synthetic elements within the cells dictating how many layers are formed. After mechanical disruption, the cells re-assembled into their layered structures, mimicking the ability of tissues to regenerate after injury. Incorporation of a lateral inhibition element enabled bifurcation and self-organization of cells originating from a single genotype, while programming subsets of cells to express different cadherins led to the formation of asymmetric structures. Such engineered self-organizing systems could provide insights into the origins of multicellularity, as well as be used to develop new tissue-like biomaterials. *CD*

https://doi.org/10.1038/s41589-018-0124-2

UBIQUITINATION

Catch and identify your prey

Nat. Struct. Mol. Biol. **25**, 631–640 (2018)

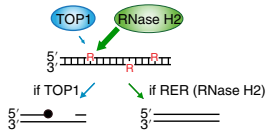
An antibody recognizing lysine-diGly-containing peptides formed during trypsin digestion of ubiquitinated proteins is commonly used to enrich ubiquitinated peptides for mass spectrometry analysis of ubiquitination sites. However, N-terminal ubiquitination cannot be detected with this antibody, and the two ubiquitin-like modifiers NEDD8 and ISG15 also yield lysine-diGly peptides after tryptic digestion, which limits the specificity of this approach. Akimov et al. overcame these limitations by raising a monoclonal antibody, UbiSite, which is specific for a 13-residue peptide formed during the digestion of ubiquitinated proteins with endoproteinase LysC. In the UbiSite workflow, proteins extracted from cultured cells or tissues are digested with LysC, enriched with the UbiSite antibody and analyzed by LC–MS/MS either directly or after additional trypsin treatment. The authors analyzed the effect of proteasome inhibitors on the ubiquitinomes of two human cell lines and identified more than 63,000 unique ubiquitination sites in over 9,200 proteins. Furthermore, N-terminal ubiquitination of more than 100 proteins was detected, which was also verified by mutagenesis studies. UbiSite improves the specificity of ubiquitinome analysis and might also be a useful tool to study the function and regulation of N-terminal ubiquitination. *KK*

https://doi.org/10.1038/s41589-018-0125-1

DNA DAMAGE REPAIR

A ribonucleotide trap

Nature **559**, 285–289 (2018)



Credit: *Nature*

Poly(ADP-ribose) polymerase inhibitors (PARPi) are extensively explored as tumor-targeted therapies for homologous recombination (HR)-deficient cancers because of the observed synthetic lethality between PARP inhibition and *BRCA1/2* mutations. The cytotoxicity of PARPi is mainly caused by the trapping of PARP1 on DNA, thus interfering with DNA duplication. To catalog cellular PARPi resistance genes, Zimmermann et al. utilized a CRISPR dropout screen approach and identified components of the RNase H2 enzyme complex as top hits. The subsequent mechanistic study revealed that the protective effect of RNase H2 was mediated by its ability to remove genome-embedded ribonucleotides without affecting HR efficiency. In addition, depleting cellular TOP1, an enzyme that can also cleave DNA-embedded ribonucleotides, reduced the DNA damage induced by the absence of RNase H2 and restored the resistance to PARPi in RNase-H2-null cells. These results suggested that TOP1-mediated ribonucleotide processing leads to DNA lesions that recruit PARP1, sensitizing RNase-H2-deficient cells to PARP trapping. This study reveals how DNA-embedded ribonucleotides cause DNA damage and PARPi sensitivity and lays a mechanistic foundation for the potential clinical use of PARPi in patients with RNase H2 deficiency. *YS*

https://doi.org/10.1038/s41589-018-0127-z

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