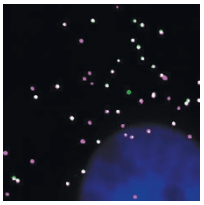
NUCLEIC ACIDS

mRNAs get a TREAT

Mol. Cell. doi:10.1016/j.molcel.2017.09.030 (2017)



ELSEVIER

Cellular mRNA levels are controlled by modulating intrinsic synthesis and degradation pathways in response to changes in gene expression and environmental conditions. Cytoplasmic mRNAs undergo decay by removal of the protective 5′ cap and 3′ polyadenine tail followed by degradation by the 5′-3′exonuclease Xrn1. Monitoring Xrn1-mediated degradation in real time is complicated, because decay intermediates exist only transiently. Inspired by the identification of pseudo-knot (PK) structures in flaviviruses that are resistant to Xrn1-mediated cleavage, Horvathova *et al.* developed a fluorescent biosensor called 3′-RNA end accumulation during turnover (TREAT) by placing two viral PKs between the PP7 and MS2 RNA stem loops, protecting MS2, but not PP7, from Xrn1-mediated degradation. This biosensor enables the monitoring of RNA degradation by measuring the ratio of intact (PP7 and MS2) versus degraded products (MS2) in fixed cells using smFISH probes targeted to the PP7 and MS2 RNA and in live cells by labeling the PP7 and MS2 coat proteins with fluorescent proteins. The TREAT sensor revealed that addition of translation inhibitors such as puromycin or cyclohexamide increased RNA stability and that cytoplasmic degradation does not occur in processing bodies (P-bodies). Exciting opportunities are now possible for coupling TREAT with existing methodologies for single-molecule imaging of transcription and translation to visualize gene expression with unprecedented detail. *GM*

PEPTIDE DESIGN

Hacking hemagglutinin

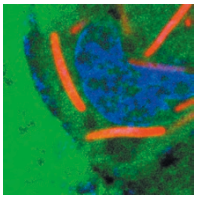
Science **358**, 496–502 (2017)

The major glycoprotein hemagglutinin (HA) binds to sialylated receptors of the host cell, enabling endocytotic uptake of the influenza virus. Acidification in late endosomes triggers a conformational change in the stem region of the HA homotrimer, leading to virus and host cell-membrane fusion. Currently, none of the influenza drugs approved worldwide target HA. Kadam *et al.* designed inhibitory peptides based on complementarity determining region (CDR) loops of broadly neutralizing antibodies (bnAb) that bind the conserved HA stem and block the HA conformational changes required for membrane fusion. Starting with linear peptides, the authors varied their length and sequence, constrained the peptide conformation by cyclization and characterized HA subtype binding specificities and affinities. They then introduced nonproteinogenic amino acids to further increase HA binding affinity. The optimized cyclic peptides have nanomolar affinities and were effective in virus neutralization assays. The cyclic-peptide-bound HA crystal structures revealed that the peptides bind in a manner very similar to the bnAb CDR loops. The structures that were determined at low pH, as well as biochemical experiments, showed that the peptides inhibit viral fusion by stabilizing the HA prefusion state. The cyclic peptides are stable in plasma and not cytotoxic, which corroborates their potential for influenza drug development. *KK*

HOST–PATHOGEN INTERACTIONS

A ubiquitin defense

Cell Host Microbe **22**, 507–518 (2017)
Nature doi:10.1038/nature24467 (2017)



NATURE

Cell-autonomous innate immune functions, such as recruitment of a family of interferon-stimulated GTPases, GBPs, to the surface of intracellular bacteria, protect mammalian cells from invading pathogens. Two studies now establish that the surface of the intracellular pathogen *Shigella flexneri* is coated with host GBPs. Explaining how *S. flexneri* can persist intracellularly, these studies showed that GBP1 was degraded by proteasomes upon *S. flexneri* infection and that this degradation was dependent on a type III secretion system (T3SS). Screening of a transposon-insertion library identified a mutation in the gene encoding the T3SS effector and the E3 ligase IpaH9.8 that failed to cause GBP1 degradation. Wandel *et al.* found that upon interferon stimulation, *S. flexneri* bacteria could not produce actin tails, which are required for bacterial motility and for transmission between host cells, and that the bacteria were decorated with polyubiquitin chains, as well as GBPs. Both studies showed that IpaH9.8 mutant bacteria maintained the GBP coat within host cells and validated IpaH9.8-mediated GBP ubiquitylation *in vitro* and proteasomal degradation in cells. Functionally, Wandel *et al.* found that GBPs restrict actin-driven motility, and Li *et al.* showed that GBPs reduce proliferation and bacterial load; both can be reversed by IpaH9.8. Finally, Li *et al.* found that in a mouse infection model, IpaH9.8 mediates degradation of GBPs and infectivity of *S. flexneri*. These results define a clever bacterial defense against a host-derived effector mechanism designed to thwart intracellular bacteria. *MB*

ENZYMOLOGY

I want my cluster back

Science **358**, 373–377 (2017)

Lipoic acid is a redox-active cofactor in various multienzyme complexes. During the biosynthesis of lipoic acid, lipoyl synthase (LipA) catalyzes the introduction of two sulfur atoms on the aliphatic chain by sacrificing one of its own two [4Fe–4S] clusters. Without a means to replace the damaged cluster, LipA catalyzes only a single turnover *in vitro*, and the mechanism that restores the cluster *in vivo* is not understood. Now, McCarthy and Booker have identified the *Escherichia coli* iron–sulfur cluster carrier protein NfuA to be capable of reconstituting this auxiliary cluster of LipA and restoring its catalytic activity. LipA and NfuA form a complex *in vitro*, enabling multiple turnovers by LipA. Isotopic tracing with ^{34}S also indicated that NfuA directly transfers a new intact [4Fe–4S] cluster to LipA, rather than repairing the damaged one, and that LipA can donate not only two, but all four, of the sulfur atoms from its auxiliary cluster to the lipoic acid product. In *E. coli*, the absence of NfuA can be compensated by a secondary iron–sulfur supply pathway involving IscU, and IscU can likewise restore LipA activity *in vitro*. However, mammals lacking the homolog of NfuA exhibit lipoyl cofactor deficiency, which may now be explained by this protein's role in reconstituting LipA activity. *CD*

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