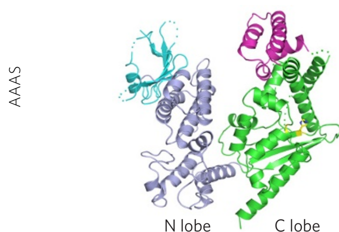
MICROBIOLOGY

Trapping Rac1

Science **358**, 528–531 (2017)



AAAS

Vibrio cholerae and other pathogenic bacteria encode multifunctional autoprocessing repeats-in-toxin (MARTX) proteins that insert into the host cell membrane and release their multiple effector domains into the cytoplasm. One of these MARTX effectors, the Rho GTPase inactivation domain (RID), decreases the amount of active GTP-bound Rho GTPases in host cells through a previously unknown mechanism. Zhou *et al.* determined that *V. cholerae* RID bound to Rac1, a Rho GTPase family member, trapping it at the cell membrane; a gel band shift indicated a post-translational modification of Rac1. The RID crystal structure revealed a bipartite structure with a membrane-binding N lobe and a catalytic C lobe similar to the human fatty acyltransferase HRASLS3. In line with fatty acyltransferase activity, RID also bound palmitoyl coenzyme A. Click chemistry and *in vitro* acylation assays provided further support that RID is a long-chain fatty acyltransferase and that Rac1 prenylation is a prerequisite for this modification. Mutagenesis and MS analysis revealed that RID catalyzes the N^{ε}-fatty acylation of lysines in the C-terminal polybasic region of Rac1. Fatty acylation of Rac1 inhibited its activation by guanine nucleotide exchange factors and blocked downstream signaling. These structural and functional insights into the RID N^{ε}-fatty acylation mechanism may be useful for future drug design. *KK*

RNA MODIFICATIONS

Ribosomes get decorated

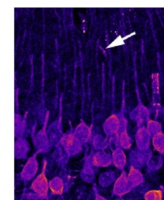
Nature doi:10.1038/nature24482 (2017)

Human ribosomes are complexes of 80 ribosomal proteins and four ribosomal RNAs (rRNAs) that are post-transcriptionally modified, primarily by 2′-OH ribose methylation and the conversion of uridines into pseudouridines (ψ). The functional consequences of rRNA modifications include regulation of RNA folding and stability, modulation of the activity of ribosome-targeting antibiotics, and dysregulation of protein synthesis as occurs in cancer. To begin to assess the role of specific modifications on ribosome function, Natchiar *et al.* mapped rRNA modifications onto the human 80S ribosome structure using cryo-electron microscopy to address their 3D environment. The authors found 136 individual rRNA modifications, mostly clustered in the interior of the ribosome at functional centers such as the peptide exit tunnel. Fifty-one of these are base modifications other than 2′-O-methylations or ψ, including several unusual modifications at nucleotide oxygen positions. Fifty-two modifications were found at unique sites not predicted from biochemically established databases, which might possibly affect translation and may be unique to the cancer cells used for the structural analysis. The authors also solved the structures of human ribosomes with three different antibiotics, showing that these inhibitors are in direct contact with or in close proximity to rRNA modification sites. These findings suggest that rRNA modification affects protein synthesis, recognition of inhibitors and tRNAs, and may act as markers of dysregulated ribosomes. *MB*

NEUROBIOLOGY

Defining your territory

Nat. Biotechnol. doi:10.1038/nbt.4016 (2017)



NAT. BIOTECHNOL.

Neurons undergo rapid alterations in gene and protein expression in response to changing environmental conditions. However, detecting these changes in an *in vivo* environment can be technically challenging. Alvarez-Castelao *et al.* developed a bioorthogonal amino acid tagging approach utilizing methionyl-tRNA synthetase (MetRS) with an expanded amino acid binding site (L274G) that loads the noncanonical amino acid azidonorleucine (ANL) and directs ANL site-selectively into nascent proteins. The authors generated a transgenic mouse line expressing MetRS L274G that was responsive to neuronal Cre recombinases such as CaMK2a-Cre or GAD2-Cre, which are expressed in excitatory and inhibitory neurons, respectively. The system enabled proteomic analysis of nascent protein production in these distinct neuronal populations, and any other Cre-addressable cell population, by treating the metabolically labeled proteins with a cleavable biotin alkyne tag and performing affinity purification combined with LC–MS/MS. Alvarez-Castelao *et al.* compared the hippocampal proteomes in response to changes in sensory cues by housing mice in a standard cage or an enriched environment.They identified 225 proteins in excitatory hippocampal neurons that were differentially regulated and that were enriched for neuronal and synaptic function. Altogether, the use of the MetRS unnatural amino acid tagging approach offers exciting opportunities to reveal proteome changes in a cell-type-specific manner. *GM*

RESISTANCE MECHANISMS

Watering down a warhead

J. Am. Chem. Soc. doi:10.1021/jacs.7b09971 (2017)

The natural product colibactin, biosynthesized by strains of *Escherichia coli* linked to colorectal cancer, contains an electrophilic cyclopropane moiety that is critical for its genotoxic activity *in vitro*. *E. coli* use several self-immunity strategies to protect themselves against the activity of colibactin, among them the resistance protein ClbS. Tripathi *et al.* have now determined that ClbS provides this protection by catalyzing hydrolysis of colibactin's cyclopropane warhead. ClbS exhibits selectivity for electrophilic colibactin analogs, as it does not hydrolyze an alternatively cyclized and stable aromatic scaffold. Although ClbS was initially identified through labeling with an activity-based probe, the crystal structure of ClbS revealed that the cysteine residue alkylated by the probe is not actually located in the active site of the enzyme and is dispensable for hydrolytic activity. ClbS also lacks the metal-binding motif found in other members of its superfamily, harboring instead a tyrosine residue in place of the metal ion. Computational docking of the ClbS substrate into the active site placed the cyclopropane moiety in proximity to this tyrosine residue, and its mutation to phenylalanine abolished the hydrolytic activity of ClbS, implicating tyrosine as a catalytic residue. The identification of ClbS as a cyclopropane hydrolase provides insights into the resistance and toxicity mechanisms of colibactin and could be useful in developing treatment strategies. *CD*

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