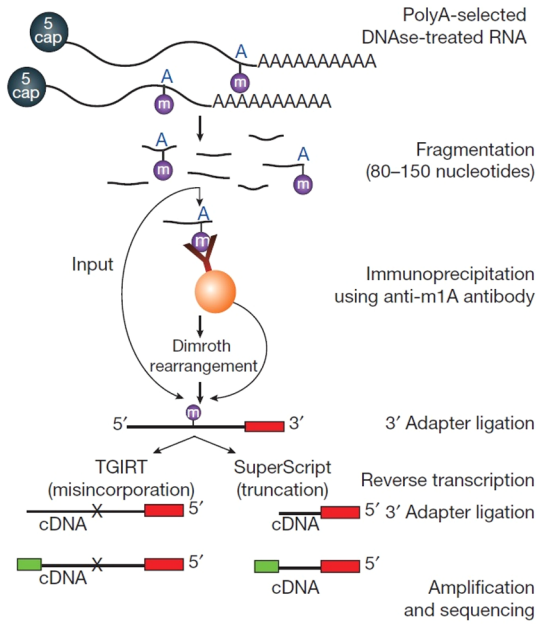
ACS chemical biology

Spotlight

Cite This: ACS Chem. Biol. 2017, 12, 2919−2921

POST-TRANSCRIPTIONAL MODIFICATION TRACKED AT SINGLE-BASE RESOLUTION



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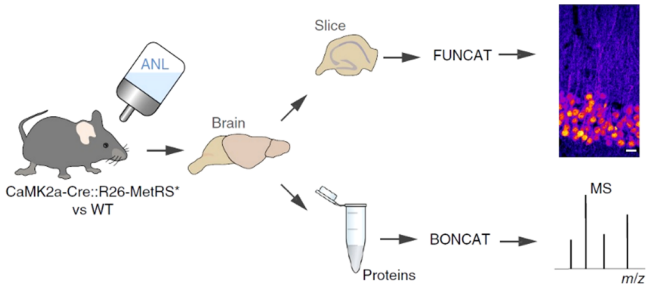
RNA molecules are mainly comprised of A, U, C, and G nucleotides, the nucleobases of which participate in intramolecular hydrogen bonds that pin RNA strands into complex three-dimensional structures with diverse functions. RNA nucleobases frequently undergo post-transcriptional modification such as alkylation, which changes nucleotide structure and/or charge and in turn affects RNA secondary structure and interactions with RNA-binding biomolecules. For example, when reverse-transcriptase polymerases, which use RNA as a template for creating complementary DNA (cDNA) strands, encounter post-transcriptional modifications that disrupt Watson−Crick base-pairing, the reverse-transcriptase may incorporate the incorrect nucleotide into the growing DNA chain or prematurely terminate reverse transcription, producing errant or truncated cDNAs, respectively.

Researchers led by Schraga Schwartz of the Weizmann Institute of Science took advantage of this interference with reverse-transcription to map the abundance and sequence context of a particular modification (N^1-methyladenosine, m^1A), in mRNAs derived from various cell types, compartments, and stages of development (*Nature* **2017**, *551*, 251−255). The research team extracted RNA from cells, specifically enriching mRNAs by targeting their polyadenylated tails, and cleaved the RNA into ∼100 nucleotide fragments. From this pool, transcripts that bound to m^1A-specific antibodies were immunoprecipitated and subjected to reverse-transcription with either TGIRT or SuperScript polymerases, which led to misincorporation or truncation at m^1A sites, respectively.

By comparing the resulting cDNA sequences with those derived from control samples in which immunoprecipitation had been skipped or in which N^1-methylations had been removed *via* Dimroth rearrangement (a chemical reaction that converts m^1A into m^6A and as a consequence eliminates the m^1A reverse-transcriptase signature), the research team was able to pinpoint the relative abundance and exact location of m^1A in mRNA. This information enabled the team to further identify enzymes involved in N^1-methylation of mRNA as well as propose the role of this particular post-translational modification in tissue development.

Heidi A. Dahlmann

SPECIFIC CELL TYPES METABOLICALLY LABELED *IN VIVO*



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Complex organisms possess tissues, organs, and organ systems composed of diverse types of cells. The variations between cell types stem directly from differential protein expression, with each cell type's proteome contributing to its function (or dysfunction) within the organism. To characterize protein expression in different types of cells, researchers have traditionally needed to carefully dissect target cells out of tissue samples before carrying out proteomics analyses. However, this laborious process may now become avoidable thanks to a newly demonstrated method for labeling proteins in specific cell types *in vivo*, recently reported by Erin M. Schuman of the Max Plank Institute and co-workers (*Nat. Biotech.* **2017**, DOI: 10.1038/nbt.4016).

The research team generated transgenic mice to express a mutant methionyl-tRNA synthase (MetRS) in specific neuronal cell types. The mutant MetRS was designed to incorporate a non-natural amino acid surrogate, azidonorleucine (ANL), in place of methionine during tRNA synthesis. The ANL-charged tRNA molecules would then be accepted by ribosomes during translation, allowing ANL to be incorporated into growing

Published: December 15, 2017

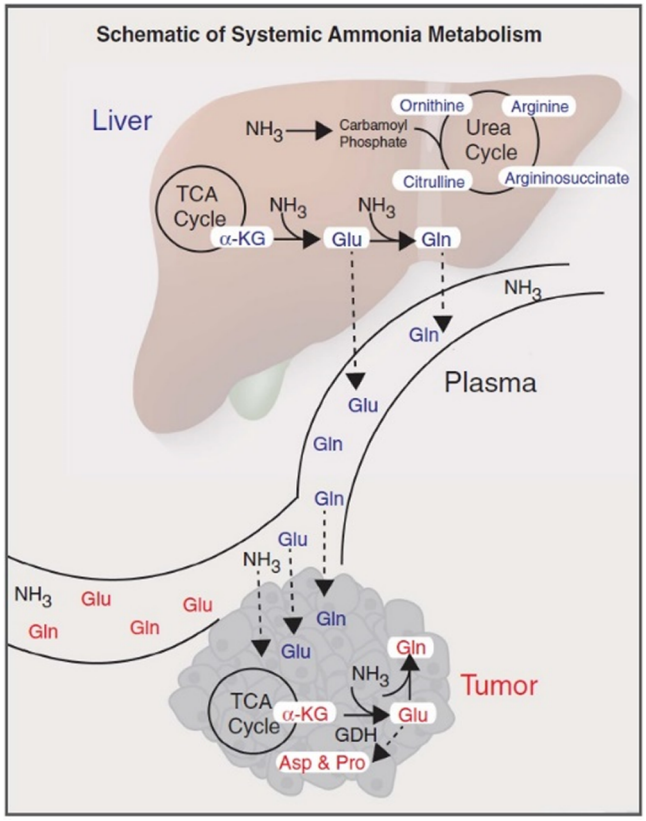
DOI: 10.1021/acschembio.7b01040
ACS Chem. Biol. 2017, 12, 2919−2921

peptide chains. Peptides containing ANL, which has an azide functional group that participates in bio-orthogonal Click reactions, could then be fluorescently tagged to enable visualization of nascent proteins directly in specific cell types within a tissue sample, or they could be processed by mass spectrometry to determine the exact identities of the nascent proteins.

Using this technique, Schuman and co-workers labeled proteins produced in excitatory principal neurons and Purkinje neurons in different strains of ANL-supplement mice, which allowed them to compare protein expression across the different cell types as well as analyze protein regulation in mice exposed to different levels of sensory stimulation in their environment. The authors note that their metabolic labeling approach could be extended to other cell types in which mutant MetRS expression can be selectively induced.

Heidi A. Dahlmann

BREAST CANCER TUMORS FEED OFF AMMONIA WASTE



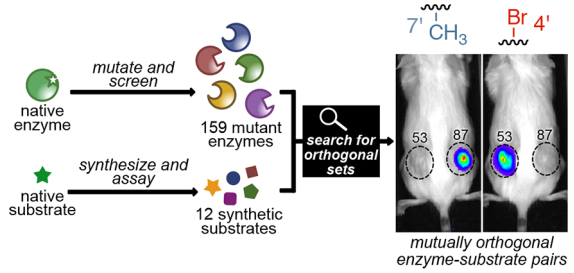
From Spinelli, J. B., *et al.*, *Science*, **2017**, *358*, 941−946. Reprinted with permission from AAAS.

Rapidly proliferating cells, such as cancer cells, require large amounts of nutrients to support cell growth and division, which in turn leads to increased production of metabolic waste. In tumors, this creates an interesting paradox: tumors are typically poorly vascularized, meaning they have relatively few blood vessels available for bringing in nutrients and carrying away waste. Under these conditions, potentially toxic metabolic byproducts eventually accumulate in the tumor microenvironment. One such metabolic byproduct that is normally considered toxic is ammonia, which is generated during glutamine and asparagine catabolism, cysteine synthesis, and salvage nucleotide metabolism. In normal tissues, ammonia is exported by cells and transported through the bloodstream to the liver, where it is incorporated into urea prior to excretion. In contrast, poorly vascularized tumors have now been discovered to take their ammonia waste and recycle it to their advantage.

Researchers led by Marcia C. Haigis of Harvard Medical School performed extensive metabolic tracing analyses to determine ammonia's fate in tumor cells (*Science* **2017**, *358*, 941−946). The team supplemented breast cancer cells with ammonia or ammonia-producing precursor substrates labeled with the rare ^{15}N isotope, which is heavier than the most naturally abundant ^{14}N isotope, allowing the team to use mass spectrometry to identify metabolites into which ammonia was incorporated. They found that most of the assimilated ammonia was incorporated by a glutamate dehydrogenase-catalyzed reductive amination of α-ketoglutarate to form glutamate, an intriguing result considering that glutamate dehydrogenase under normal conditions primarily carries out the reverse reaction, oxidative deamination. The reductive amination product, glutamate, could then be converted into other amino acids, ultimately enabling tumor cells to scavenge their own waste to fuel further cell growth.

Heidi A. Dahlmann

TINKERING WITH BIOLUMINESCENCE TOOLS



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In the 20th century, technologies to look inside of living cells and whole organisms greatly expanded our grasp of basic biology and medicine. Over these past two decades, dramatic improvements in molecular tools and imaging technologies delivered better snapshots and movies, driving new discoveries. One colorful example is the ever expanding rainbow of engineered fluorescent proteins, allowing cell biologists to simultaneously watch the localization of multiple proteins in a cell. Bioluminescent imaging with luciferase offers another powerful option to look inside organisms since an excitation light source is not required, but so far it has lagged behind fluorescence techniques when it comes to seeing multiple targets at once.

Recently, Rathbun *et al.* (*ACS Cent. Sci.* **2017**, DOI: 10.1021/acscentsci.7b00394) pursued this challenge by screening mutant luciferases with chemically modified substrates in a hunt for orthogonal pairs with activity and specificity. They gathered 12 variants of the natural substrate, luciferin, each harboring an additional moiety at a key position known to bind near the firefly luciferase backbone. In a parallel manner, a library of mutant luciferase enzymes was screened by a bacterial colony assay to identify those that are functional with a modified luciferin. In all, 159 enzymes were screened for activity with the 12 substrates, and a custom *in silico* analysis identified

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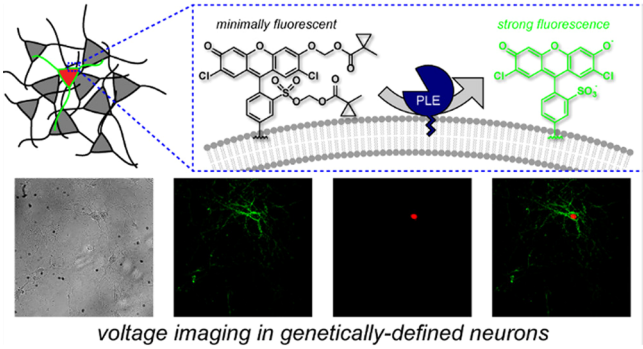
the best orthogonal pairs for additional experiments. Dozens of hits were characterized by *in vitro* assays with pairs showing the best specificity performance carried forward for *in vivo* imaging experiments with mice. The result was several new luciferase mutants harboring preferential activity for a particular flavor of modified luciferin. Since the synergy between activity screening and computational analysis proved fruitful even with a limited number of enzyme−substrate combinations, future optimizations could expand the bioluminescent toolkit even further for multicomponent analysis.

Jason G. Underwood

DYE STRATEGY MAPS VOLTAGE IN NEURONS



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Neuroscientists would like to map neuronal voltage changes in space and observe them over time. However, widely used, voltage-sensitive chemical dyes cannot target specific cell types, limiting their usefulness in intact brains. In a new study, Liu *et al.* present a hybrid approach that uses a modified chemical dye that is minimally fluorescent until paired with a genetically encoded enzyme that unmasks it on cells of interest (*J. Am. Chem. Soc.* **2017**, DOI: 10.1021/jacs.7b07047). This is the first time that researchers have demonstrated a fluorogenic, functional imaging strategy in brain cells.

Evan Miller's laboratory has been developing VoltageFluor dyes, molecules that associate with cell membranes and fluoresce in response to voltage changes within them. To dim their fluorescence, the researchers designed and synthesized two different VoltageFluor dyes that included bulky, hydrolytically stable acetoxymethyl ethers on the phenolic oxygen. However, porcine liver esterase (PLE) can lop off these groups, so they modified this enzyme to genetically encode it within cells of interest and target it to the cell membrane.

First, they tested the dyes as PLE substrates *in vitro* and then studied them with modified PLE expressed on the surface of eukaryotic HEK cells. The fluorescence change is proportional to the voltage applied (approximately 20% $\Delta F/F$ per 100 mV). They confirmed that PLE only unmasks dye molecules within the membrane of cells that express the enzyme and not on neighboring cells.

Then, they tested this strategy in rat hippocampal neurons. Although they were able to observe voltage-based neuronal responses modulated by serotonin, the contrast was not as high as in HEK cells or as current genetically encoded sensors. The researchers expect that boosting expression levels of PLE and further tweaks to the dyes will improve cellular contrast and make these dyes suitable for routine use *in vivo*.

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DOI: 10.1021/acschembio.7b01040
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