tion of the claim of "Super AIDS" in 2005, when alarm was raised over a rapidly progressing, multidrug-resistant HIV infection found in New York (*10*) that was ultimately restricted to a single individual.

These findings are relevant to the COVID-19 pandemic. Although it is certainly possible that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) will evolve toward a more benign infection (*11*), like other "common cold" coronaviruses, this outcome is far from preordained. At the beginning of the COVID-19 pandemic, there was an underappreciation of the rapidity with which selection would lead to changes in transmissibility and virulence (*12*). But the ultimate outcome depends on whether and how SARS-CoV-2 transmission and virulence are linked. SARS-CoV-2 variants demonstrate that this virus is repeatedly evolving to be more transmissible, and not all of these adaptive variants are demonstrably more virulent. However, the Delta variant that dominated global cases in late 2021 shows how SARS-CoV-2 could evolve to be both more transmissible and more virulent (*13*). The Omicron variant is more transmissible, but whether it is more or less virulent in immunologically naïve individuals is unclear. Immune evasion, receptor binding efficiency, and tissue tropism may contribute to the evolution of virulence (*14*, *15*). Deciphering the mechanisms of SARS-CoV-2 virulence and its relationship with transmission and immunity will be essential to understand how and why its virulence may evolve. But the HIV and SARS-CoV-2 pandemics show how viruses can and will evolve higher virulence when favored by natural selection. ■

REFERENCES AND NOTES

1. J. L. Geoghegan, E. C. Holmes, *Nat. Rev. Genet.* **19**, 756 (2018).
2. C. Wymant *et al.*, *Science* **375**, 540 (2022).
3. P. J. Kerr *et al.*, *Viruses* **7**, 1020 (2015).
4. C. Fraser *et al.*, *Science* **343**, 1243727 (2014).
5. C. Fraser, T. D. Hollingsworth, R. Chapman, F. de Wolf, W. P. Hanage, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 17441 (2007).
6. F. Blanquart *et al.*, *eLife* **5**, e20492 (2016).
7. J. T. Herbeck *et al.*, *AIDS* **26**, 193 (2012).
8. J. O. Wertheim *et al.*, *Nat. Commun.* **10**, 5788 (2019).
9. A. M. Oster, A. M. France, J. Mermin, *JAMA* **319**, 1657 (2018).
10. L. M. Crawley, *JAMA* **7**, 790 (2005).
11. J. S. Lavine, O. N. Bjornstad, R. Antia, *Science* **371**, 741 (2021).
12. N. D. Grubaugh, M. E. Petrone, E. C. Holmes, *Nat. Microbiol.* **5**, 529 (2020).
13. A. Sheikh, J. McMenamin, B. Taylor, C. Robertson, *Lancet* **397**, 2461 (2021).
14. M. Diamond *et al.*, *Research Square* 10.21203/rs.3.rs-1211792/v1 (2021).
15. B. Meng *et al.*, *bioRxiv* 10.1101/2021.12.17.473248 (2021).

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EPIGENETICS

The adenine methylation debate

N^6-methyl-2′-deoxyadenosine (6mA) is less prevalent in metazoan DNA than thought

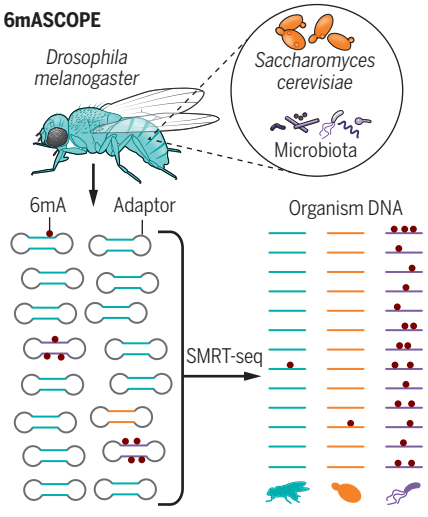
By **Konstantinos Boulias**[1,2] *and* **Eric Lieberman Greer**[1,2]

Adenine methylation, forming N^6-methyl-2′-deoxyadenosine (6mA), is a prevalent DNA modification in prokaryotes and has recently been proposed to exist in multicellular eukaryotes (metazoans) to regulate diverse processes, including transcription, stress responses, and tumorigenesis. However, the existence of 6mA, and therefore its biological importance, in metazoan DNA has been debated by recent studies, which have either detected 6mA at much lower abundances than initially reported or failed to detect 6mA at all. On page 515 of this issue, Kong *et al.* (*1*) report the development of 6mASCOPE, a quantitative method that deconvolutes 6mA in samples of interest from contamination sources. They detected low amounts of 6mA in fruit flies (*Drosophila melanogaster*), plants (*Arabidopsis thaliana*), and humans, which suggests that 6mA is much less abundant in these organisms than previously thought. These data suggest that a reassessment of 6mA in eukaryotic DNA is warranted.

The discovery of 6mA in multicellular eukaryotic DNA (*2*–*4*) was facilitated by the development of highly sensitive detection and mapping methodologies. These include ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS), which has a detection limit of 0.1 to 1 parts per million (ppm) (*5*), and single-molecule real-time sequencing (SMRT-seq), a long-read DNA sequencing technique that maps methylated bases by quantifying rates of incorporation of complementary bases, which are altered when bases are modified (*6*). However, these methods have limitations: UHPLC-MS/MS cannot discriminate the source of 6mA, which becomes problematic when 6mA is of low abundance in the organism compared with the abundance in bacterial contaminants (*7*). Moreover, long-read sequencing methods, such as SMRT-seq, are error prone, and SMRT-seq requires high sequencing depth and loses accuracy when 6mA is lower than 10 ppm (*7*, *8*). Because of these limitations, several laboratories have been unable to detect 6mA, or they have detected 6mA at substantially lower concentrations in metazoan genomes (*7*–*10*), which has led some to question whether 6mA is a directed DNA modification in metazoans. Kong *et al.* developed 6mASCOPE, a SMRT-seq analysis method that quantitatively de-

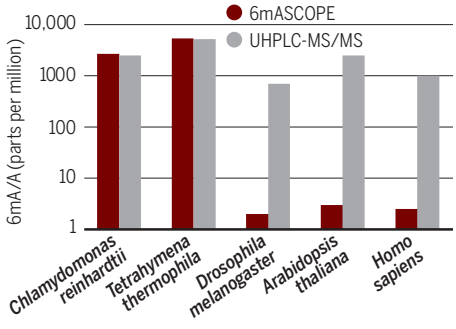
Organisms with adenine methylation

To quantify the amount of N^6-methyl-2′-deoxyadenosine (6mA) present in genomic DNA, single-molecule real-time sequencing (SMRT-seq) data are analyzed with 6mASCOPE. In 6mASCOPE, small DNA fragments are produced, adaptors are added, and high-coverage SMRT-seq is performed. 6mASCOPE is a reference-free analysis method that deconvolutes SMRT-seq data to identify the source of 6mA.



The abundance of 6mA

Previously, ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) identified 6mA in prokaryotes and metazoans. However, analysis with 6mASCOPE found that 6mA was overestimated in metazoans owing to contamination with DNA from microbiota or food.



GRAPHIC: KELLIE HOLOSKI/*SCIENCE*

termines in which species 6mA is present, enabling discrimination between 6mA in the metazoan genome and that in contaminating microorganisms (see the figure).

Existing SMRT-seq methods compare the interpulse duration [(IPD) the time between successive base additions, which is altered by DNA modifications] of native template with the reference genome, ignoring contaminating DNA with abundant 6mA. Kong *et al.* overcome this limitation by devising a reference-free approach. By using the long-read sequencing to exclusively sequence short (200 to 400 base pairs) DNA sequences, each molecule is heavily resequenced, which leads to higher-confidence circular consensus sequence (CCS) base-calling accuracy. A metagenomic analysis allows for CCS reads to be mapped to both the genome of interest and to potential contamination sources by using a comprehensive set of genomes, including those from microbiota. The 6mA/A ratios were estimated using a machine learning model trained with a broad range of 6mA content. As a proof of principle, the authors performed 6mASCOPE on two unicellular eukaryotes with high amounts of 6mA, *Chlamydomonas reinhardtii* (*11*) and *Tetrahymena thermophila* (*12*). They confirmed high 6mA in these protists and further refined the methylation motif (VATB: V = A, C, or G; B = C, G, or T) and preference of 6mA to occur in specific locations in the linker regions between nucleosomes.

Kong *et al.* next applied 6mASCOPE to *D. melanogaster*, *A. thaliana*, and *Homo sapiens*—three multicellular eukaryotes with reported high 6mA abundances [~700 ppm for *D. melanogaster* embryos (*2*), 2500 ppm for *A. thaliana* seedlings (*3*), and 500 to 1000 ppm for *H. sapiens* lymphocytes (*13*) or primary glioblastomas (*14*)]. They found that bacteria in the gut of *D. melanogaster* or in the soil of *A. thaliana* samples, which made up a very small amount of the mapped reads, accounted for the majority of 6mA quantified by UHPLC-MS/MS. This led to 6mA abundance in *D. melanogaster* and *A. thaliana* genomes being quantified at ~2 or 3 ppm (near the limit of detection). These findings are bolstered by previous work that demonstrated that nematode worms (*Caenorhabditis elegans*) have substantially lower 6mA abundance (0.1 to 3 ppm) than previously estimated because of bacterial contamination in the gut and that zebrafish (*Danio rerio*) embryos have artificially increased 6mA quantifications because of bacteria adhering to the chorion membrane, which surrounds the embryo, as assessed by UHPLC-MS/MS (*7*).

6mASCOPE performed on peripheral blood mononuclear cells and two glioblastoma brain tissue samples yielded 6mA abundances of 17 and 2 ppm, respectively. A recent study suggested that 6mA is increased in mammalian mitochondrial DNA (*15*), but 6mASCOPE also failed to detect increased amounts of 6mA in the mitochondrial DNA of human HEK293 cells. Kong *et al.* confirmed earlier results (*7*, *10*) that exogenous premethylated DNA can be incorporated into eukaryotic DNA and increases 6mA content. Together, these findings challenge high 6mA abundances in multicellular eukaryotes. Instead, 6mA is likely much rarer than previously thought and is possibly variable between different tissue samples or cell lines. It is also possible that 6mA increases only under specific stress conditions (*15*).

6mASCOPE's limit of detection (~1 to 10 ppm) makes it hard to conclude whether estimated 6mA abundances of 2 to 3 ppm are real and above background. These limitations can be addressed through the development of sequencing methods that take advantage of the distinct chemistry of 6mA, similar to bisulfite sequencing for 5-methylcytosine. Additionally, future studies should combine this more-rigorous 6mASCOPE and optimized UHPLC-MS/MS methods (*7*) with a focus on stress conditions and mitochondrial DNA (*15*). Moreover, 6mASCOPE cannot discriminate potential misincorporation of either abundant messenger RNA containing 6mA or foreign methylated DNA that could be integrated into eukaryotic DNA through the nucleotide salvage pathway. Combining rigorous detection methods with the manipulations of putative 6mA-regulating enzymes and directed epigenomic editing of 6mA will help address whether rare 6mA in metazoans has a functional role in specific locations in the genome or is randomly localized as a potential by-product of misincorporation by the salvage pathway. ■

REFERENCES AND NOTES

1. Y. Kong *et al.*, *Science* **375**, 515 (2022).
2. G. Zhang *et al.*, *Cell* **161**, 893 (2015).
3. Z. Liang *et al.*, *Dev. Cell* **45**, 406 (2018).
4. E. L. Greer *et al.*, *Cell* **161**, 868 (2015).
5. W. Huang *et al.*, *RSC Adv.* **5**, 64046 (2015).
6. B. A. Flusberg *et al.*, *Nat. Methods* **7**, 461 (2010).
7. Z. K. O'Brown *et al.*, *BMC Genom.* **20**, 445 (2019).
8. K. Douvlataniotis *et al.*, *Sci. Adv.* **6**, eaay3335 (2020).
9. S. Schiffers *et al.*, *Angew. Chem. Int. Ed.* **56**, 11268 (2017).
10. B. Liu *et al.*, *Anal. Chem.* **89**, 6202 (2017).
11. Y. Fu *et al.*, *Cell* **161**, 879 (2015).
12. Y. Wang *et al.*, *Nucl. Acids Res.* **45**, 11594 (2017).
13. C.-L. Xiao *et al.*, *Mol. Cell* **71**, 306 (2018).
14. Q. Xie *et al.*, *Cell* **175**, 1228 (2018).
15. Z. Hao *et al.*, *Mol. Cell* **78**, 382 (2020).

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MATERIALS SCIENCE

An adaptive device for AI neural networks

The perovskite nickelate can transform among four different electronic components

By **Rohit Abraham John**

The human brain's ability to maneuver the avalanche of unstructured data, learn from experience, and process information with extreme energy efficiency inspires the next generation of computing technologies (*1*, *2*). Neuronal plasticity is defined as the capability of the brain to change its structure and function in response to experience. This functional and structural plasticity is what researchers are trying to achieve in the so-called "neuromorphic" circuits and computer architectures (*3*–*6*). Specific learning rules observed in biology have been faithfully replicated recently in electrical components (*7*, *8*). However, the ability for a logical device to learn and modify from experience, and to grow and shrink when required, have yet to be explicitly demonstrated. On page 533 of this issue, Zhang *et al.* (*9*) present highly plastic perovskite nickelate devices that can be electrically configured and reconfigured to become resistors, memory capacitors, artificial neurons, and artificial synapses.

The material design principle for creating reconfigurable devices is based on protonation-induced doping of nickelates such as $NdNiO_3$, or NNO. At room temperature, an ideal NNO is a correlated metal, which means that electrons would interact among themselves inside the material instead of behaving independently. Hydrogen, an electron donor, can be inserted into the NNO lattice by annealing the material in hydrogen gas while connected to a catalytic electrode. This process modifies the electrons'

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