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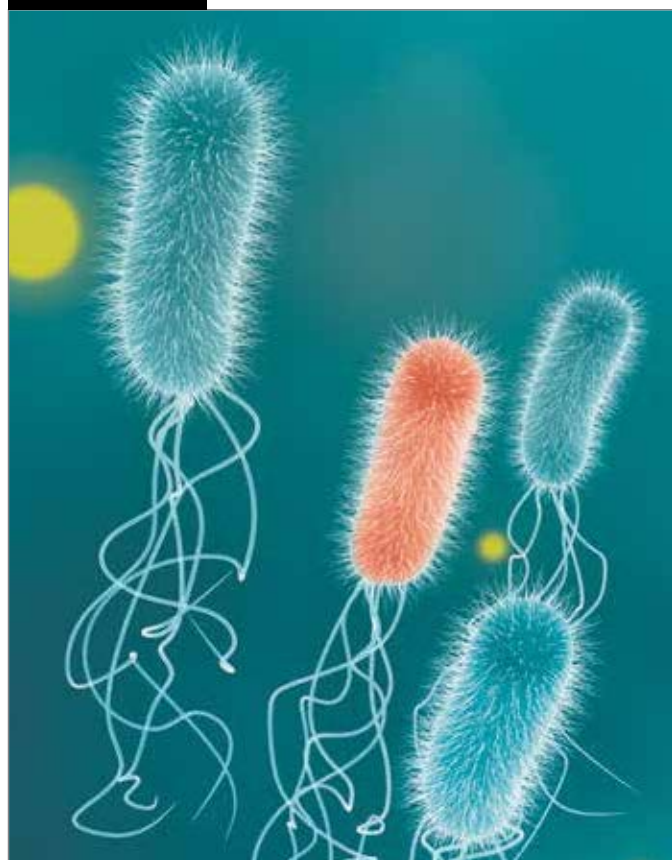
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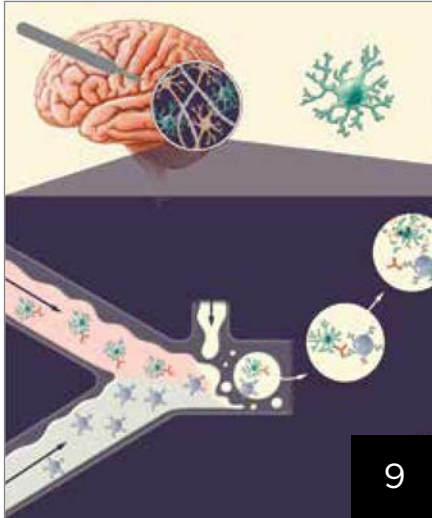
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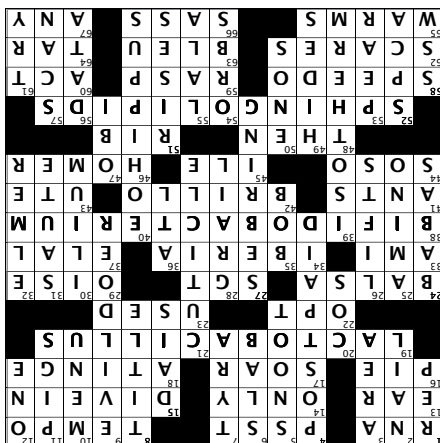


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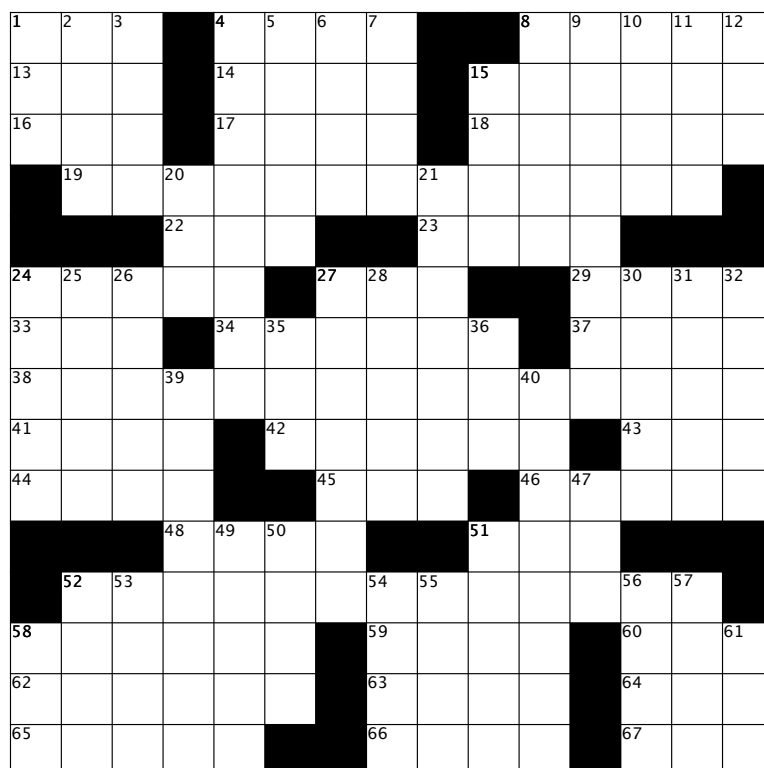
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Ten Minute Sabbatical

ACROSS

1. Strand synthesized via transcription
4. Sibillant summons
8. Small molecule used as a structural probe
13. Organ with a tectorial membrane
14. Sole
15. Enter with enthusiasm
16. Chart format commonly used to show percentages
17. Emulate a condor
18. Very, very little
19. Gut flora genus found in dairy products
22. Make a selection
23. Tapped into
24. *Ochroma pyramidale*, more familiarly
27. PFC's boss
29. River of Belgium and France
33. French friend
34. Granada hare's habitat
37. Airline that serves only kosher meals
38. Gut flora genus found in sauerkraut
41. Formic acid producers
42. Brand of scrubbing pads
43. Sport-___ (versatile vehicle, for short)
44. Not great
45. Land in la mer
46. Family of adaptor proteins that shares its name with a Greek poet
48. If-___ (hypothesis format, often)
51. Vertebrosteral bone
52. Gut flora products implicated in some metabolic disorders
58. Teensy swimsuit brand
59. Grating quality
60. Exam for high school students that has a science section
62. Fills with alarm
63. ___ cheese dressing
64. Substance that may preserve fossils
65. Increases in temperature
66. Pert response
67. Whichever



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BY STELLA ZAWISTOWSKI

DOWN

1. Drug salesperson, for short
2. Structure mostly made of keratin
3. Surface ___-to-volume ratio
4. With stamps already attached, perhaps
5. Nose, cutely
6. Lump of electrophoresis gel
7. Supermodel Banks
8. "The Selfish Gene," for one
9. Malevolent actor
10. Restaurant handout
11. Preclinical research subjects, often
12. Number of neutrons in a deuterium nucleus
15. Speaker's platform
20. Inverse of sec
21. Protective cover on plant leaves
24. Rummy desserts
25. -NH₂
26. Elevators, across the pond
27. Old Chrysler model
28. "Holy" quest of Round Table knights
30. Big pelvic bone
31. Cook in a pan
32. Name associated with glue
35. Go up and down
36. Emory's home, for short
39. Line on a climate map
40. Extinct relative of modern-day horses
47. Accessory worn with a kimono
49. Keeps out of sight
50. Enzyme that synthesizes nitric oxide
51. Comes above the horizon
52. Organization concerned with lab safety?
53. Fruit with European and Asian cultivars
54. Spherical shapes
55. ___ land (dreamlike state)
56. Stuff collected in an experiment
57. Micro-CT product
58. Direction opposite NNE
61. Make an attempt

Answer key on page 5

Using Bug Brainpower to Smell Cancer

Scientists use locust brains as living biosensors to perform cancer cell breath tests.

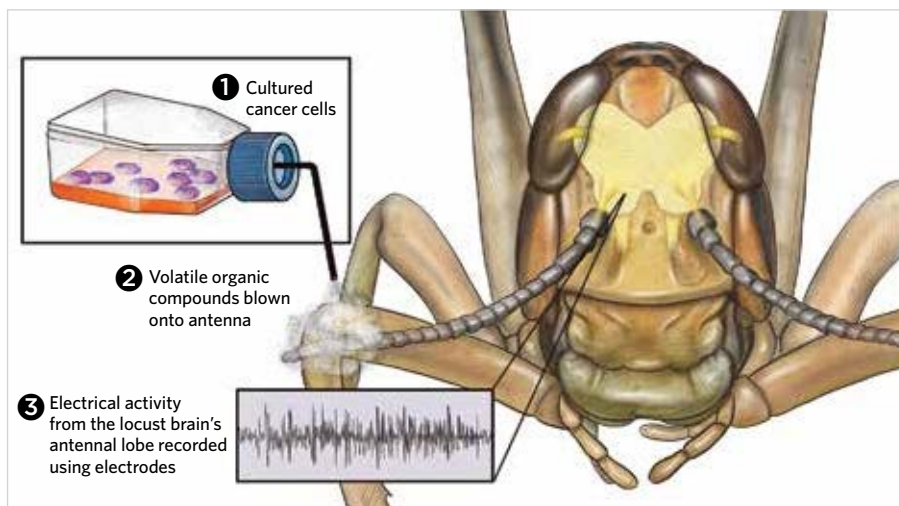
BY IRIS KULBATSKI, PhD

Fathoming what it is like to be a wild thing is beyond the limited resolution of human senses. For example, insects live in a world of smell, decoding the chemical nuances of scent that waft across their antennae. In the case of locusts—the migratory swarming form of certain asocial grasshoppers—their exquisite ability to detect and differentiate subtle odors drives their physical and communal transformation. Certain environmental conditions trigger locusts to molt and secrete pheromones that attract more locusts until billions aggregate in devastating swarms.

Recently, Debajit Saha, an assistant professor of biomedical engineering at Michigan State University, and his team tapped into the locust brain's odor-sensing circuitry to detect the scent signatures of human oral cancers. Saha previously used locusts for sniffing out bombs, making the transition to cancer detection an interesting one. "Cancer changes [cellular] metabolism and those changes are reflected in exhaled breath," Saha said.

Known as volatile organic compounds (VOCs), these unique chemical signatures are promising biomarkers—if scientists can detect them. Saha's team turned to biology to harness nature's most powerful biosensors. "Rather than back engineering a biological brain, we are forward engineering their computation," Saha said.

Saha's team intercepted the odor processing circuits in the locust brain to detect and discriminate between human oral cancer cells growing in a flask and reported their findings in *Biosensors and Bioelectronics*. They inserted electrodes into the locust brain regions that process smell. They then collected gas samples from the cell cultures of three different types of human oral cancer cells and



SCENTS AND SENSE-ABILITIES: Scientists hack the locust brain to identify the unique odor signatures of oral cancers. Researchers placed electrodes in the odor processing regions of locust brains to intercept the electrical signals produced after they puffed cancer cell gas samples over their antennae. They recorded different electrical patterns for different types of oral cancer. The researchers said that "cyborg locust" brains make powerful biological computers.

healthy human oral cells. They wafted these gas samples—each of which contained a distinct mixture of VOCs from the corresponding cells—over the locust's antennae and recorded the brain's electrical activity. After pooling the results from multiple locusts, Saha's team found that the locusts' brains not only distinguished cancer from healthy cells but also discriminated between the subtle scent fingerprints of different oral cancers.

"It is important work that adds to our field and may one day lead to actual devices for making health diagnoses," said Bruce Kimball, a chemical ecologist at the Monell Chemical Senses Center, who was not involved in this study. According to Kimball, any combination of a detector—sensory neurons, artificial sensors, or other analytical instruments—with a pattern analysis algorithm—biological brain, brain-mimicking chip, or machine learning—could eventually

help researchers cross the finish line. Given enough fine-tuning, any such system "will likely be successful at discriminating among odors associated with most health conditions."

Saha explained that the computational power of the locust biosensors requires a limited number of odor receptors to create an almost unlimited capacity to encode different chemicals. "They don't have to identify individual components; they can just take the whole mixture and create [an odor] fingerprint," Saha said. His team plans to test breath samples from human cancer patients, which have far more complex scent signatures than cultured cells. "That will be the big test."

After millennia of inflicting turmoil, locusts may finally redeem themselves in the aid of humankind. They remind us that our understanding of the world is only as accurate as the instruments of perception that we use. ■

New Hope for Epilepsy Treatment

Clinicians and researchers teamed up to investigate how inappropriate proinflammatory mechanisms contribute to the pathogenesis of drug-refractory epilepsy.

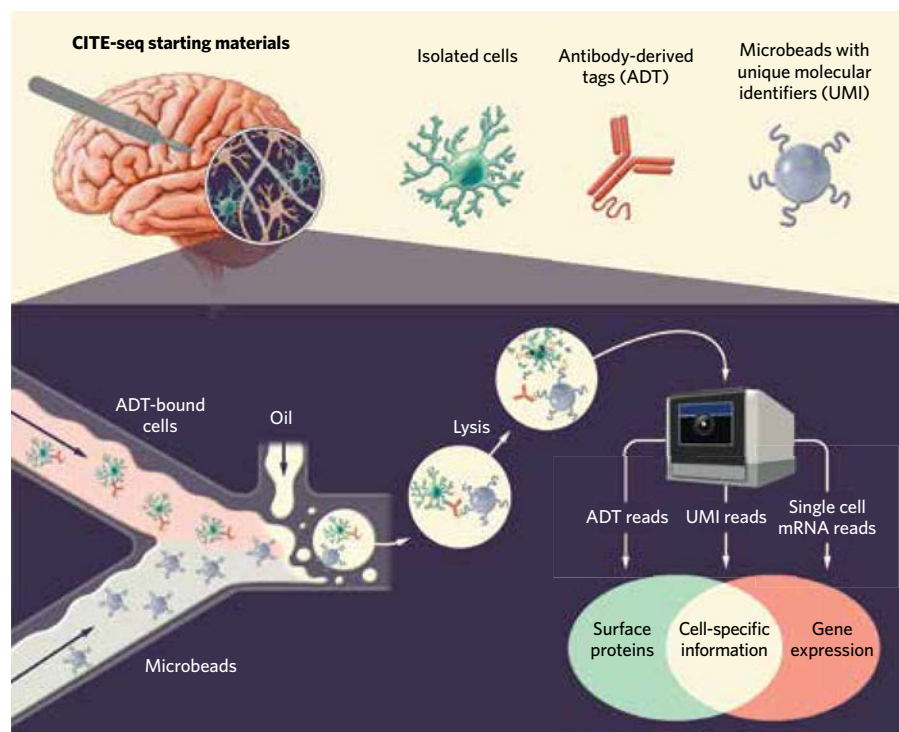
BY DEANNA MACNEIL, PhD

Doctors treat epilepsy with anticonvulsants to control seizures, but some patients do not respond to these first-line therapies. For patients with drug-refractory epilepsy (DRE) whose seizures persist after treatment with two or more anticonvulsants, clinicians must surgically remove part of the brain tissue to cure the disease.

When first-line medicines fall short, scientists examine the molecular mechanisms of a disease to understand why and to develop alternatives. At Duke-NUS Medical School and KK Women's and Children's Hospital, clinicians and researchers teamed up to investigate how inappropriate proinflammatory mechanisms contribute to DRE pathogenesis. This work builds on evidence that inflammation is associated with epilepsy. For Derrick Chan, a clinician scientist at KK Women's and Children's Hospital, this research is an extension of his clinical work. "This direction became really important because we were looking for a less invasive way to try to help all the children with drug resistant epilepsy," he said.

Chan and his team partnered with the immunology research group of fellow physician scientist, Salvatore Albani. In a study published in *Nature Neuroscience*, Chan and Albani described their efforts to understand the immunologic factors that contribute to DRE pathology. The researchers used a single-cell sequencing technique called cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) to examine surgically resected epileptic tissue from patients.

They identified cell-specific functions at single-cell resolution and differentiated resident cells from infiltrating immune cells. The researchers found that the DRE microenvironment includes acti-



CITE-SEQ DETECTS PROTEIN AND GENE EXPRESSION IN THE SAME CELL: Scientists mix single cells with ADTs and encapsulate them in droplets, along with microbeads. Cell lysis within the droplet releases mRNAs and antibody-derived tags (ADT) for microbead capture before PCR amplification, size separation, and single cell RNA sequencing (scRNA-seq).

vated microglia and other proinflammatory immune cells. "We had not expected these interactions between microglia and other immune cells, and then how these microglia become kind of a pivot to attract all of the immune cells by starting this proinflammatory milieu inside the brain," explained Pavanish Kumar, coauthor of the study.

Previous work from Albani and Chan underscored how systemic inflammation contributes to epilepsy pathogenesis, which prompted them to investigate the local inflammatory signature in brain tissue. It can be a challenge to study brain tissue, and as Albani pointed out, access

to samples is one limitation of this study. "It's not as simple to get brain tissue from live patients... so numbers are small," he explained. The researchers emphasized that future work will involve animal models to establish a robust foundation to build on.

"It's definitely a big jump forward in our field," said David Henshall, a professor of physiology and medical physics at Royal College of Surgeons, who was not involved in the study. "Perhaps we can now, based on this study, design a treatment or combination of treatments that may be more precise than what has been tried so far." ■

Integrate and Innovate with NGS and Multiomics

Researchers across disciplines combine layers of discovery obtained with accessible NGS-based multiomics approaches.

Advances in genomic technologies provide researchers with access to more complete molecular datasets than ever before. Next-generation sequencing (NGS) in particular propels both basic and translational research forward, enabling scientists to see the full picture of biology. As a result, researchers now know more about the genetics and pathways underlying disease and have databases of actionable information that can be considered for screening and therapeutic development. For instance, scientists used high throughput sequencing technologies for the cancer genome atlas (TCGA) project—a massively successful NGS-based research effort to thoroughly characterize the genome, transcriptome, epigenome, and proteome across 33 different types of cancer. The TCGA database is publicly available to all researchers, and has already led to improvements in cancer diagnostics, treatments, and prevention.^{1,2}

Analyze Any -Ome with NGS

Researchers examine different layers of biological regulation with a variety of technologies. From Sanger sequencing for characterizing mutations to quantitative polymerase chain reaction (qPCR) for quantifying specific transcripts, scientists have had the capacity to answer a number of targeted research questions. Although each of these methods remain critical in cellular and molecular biology research, scientists turn to NGS as a powerful, scalable tool for bringing thousands of these -omes together on a single platform. This scalability provides comprehensive and unbiased characterization capabilities, which enable scientists to study more than just the low hanging fruit. NGS allows researchers to make discoveries that they may not have even considered prior to experimental design.³

For example, at the start of the TCGA research projects in 2006, shotgun sequencing and microarray-based technologies led the way for profiling copy number variants, methylation, and protein expression. Over the 12-year-span of TCGA, the demand for scalable, low-cost data brought high throughput NGS to the forefront. NGS—alone and in combination with array-based methods—enabled and expanded whole genome, exome, transcriptome, and epigenome studies.¹

NGS-Based Multiomics Is the Future of Research

Researchers contributing to the TCGA have pieced together information from different -omics datasets that were collected separately. However, the simultaneous analysis of multiple -omes is crucial for researchers to gain a comprehensive view of biological complexity.⁴

Multiomics is the combined analysis of two or more -omes from the same samples. With this approach, scientists leverage the benefits of each -omic technique and answer previously unapproachable research questions. Multiomics can include any combination of genomics, transcriptomics, proteomics, and epigenomics, as



Multiomics Definition

Scientists ask and answer innovative research questions by combining -omic modalities to create something new. Multiomics can include any combination of genomics, transcriptomics, proteomics, and epigenomics, as well as intersections and extensions of these layers, such as microbiomics, metabolomics, interactomics, and immunomics.

well as intersections and extensions of these layers, such as microbiomics, metabolomics, interactomics, and immunomics.⁵

Researchers turn to multiomics for a variety of reasons. Because NGS-based multiomics characterizes thousands of targets at once across multiple layers of regulation in the same starting material, scientists maximize discovery from precious samples and reduce the need to infer connections between the cause and consequence of complex phenotypes in separate isolates.⁶

The Unique Value of Combined -Omics

From tracking cancer cell evolution to characterizing Alzheimer's hallmarks, scientists employ NGS-based multiomics for high throughput and cost-effective investigations of multifaceted biological systems.⁵

Genome and proteome: Single cell targeted resequencing and cell surface protein analysis

Researchers at Memorial Sloan Kettering Cancer Center and the University of California, San Francisco employed single cell multiomics to track the cellular evolution of myeloid cancer. With oligo-conjugated antibodies, the scientists performed high throughput single cell proteomics with DNA sequencing. They simultaneously analyzed mutations and quantified cell-surface proteins for more than 740,000 cells. This level of scale and detail allowed the researchers to connect genotype with phenotype by finely mapping the dynamics of cancer cell clonal complexity and its effect on disease progression, and by discerning which genes are disease drivers versus passengers. Specifically, by combining analysis of protein expression and mutations, the researchers were able to map both the somatic genotype and clonal architecture with immunophenotype.⁷

Transcriptome and epigenome: ChIP-seq and total RNA-seq

Although protein aggregation is a hallmark of Alzheimer's disease, therapies that target those proteins have not been effective. A group of scientists from the University of Pennsylvania aimed to understand this dilemma by examining epigenetic dysregulation

in early Alzheimer's disease with the power of multiomics. The researchers compared brain tissue from healthy individuals and people affected by Alzheimer's with total RNA-seq and epigenetic sequencing assays, including chromatin immunoprecipitation sequencing (ChIP-seq) and 5-hydroxymethylcytosine (5hmC) analysis. They found that the epigenomic landscape differs between these groups of individuals, and that protective pathways involved in healthy aging are disabled to initiate epigenetic changes that drive Alzheimer's disease. Combining epigenetic and RNA-seq methods enabled the scientists to directly measure links between gene regulation and gene expression, instead of inferring those connections.⁸

Metagenomics and transcriptome: 16S rRNA gene sequencing and tissue mRNA-seq

By taking a multiomics approach, scientists from Mayo Clinic studied the connection between the stomach microbiome and host gene expression in gastric cancer. They integrated bacterial gene sequencing and human transcriptomics of biopsy samples from healthy stomachs, as well as gastritis and cancer lesions. The researchers uncovered disease-specific connections between the host and bacteria, such as unique bacterial species linked to human genes involved in cell division. They also noted immune signatures in gastric cancer that may suggest a previously unidentified immune evasion process. Investigating 16S rRNA gene sequencing and tissue mRNA-seq in the same samples allowed the researchers to collect an unprecedented comprehensive description of microbial genomics, human transcriptomics, and immune cell factors related to gastric carcinogenesis.⁹

Transcriptome and proteome: CITE-seq

With concurrent measurements of protein and transcript levels, researchers characterize cellular heterogeneity, identify rare cell subpopulations, and detect information otherwise lost with bulk methods. A research team from the University of Minnesota profiled immune cells in the heart with such a multiomics approach, called CITE-seq (cellular indexing of transcriptomes and epitopes). CITE-seq provides an easy and comprehensive view of single cell function by combining transcriptional information that reflects cell physiology and post-transcriptional information about surface proteins. The researchers used both mRNA-seq and oligo-tagged antibodies to evaluate gene expression and protein levels simultaneously at a single cell level, and identified a heterogeneous population of macrophages that both stimulate angiogenesis and slow fibrosis.¹⁰

New Technologies Make Multiomics a Reality for More People

With cutting edge NGS technologies, multiomics is becoming more accessible to more researchers. **Illumina® NovaSeq™ X** sequencers are great examples of advanced NGS technology that brings cost-effective, large-scale datasets within reach for all researchers, and expands the scope of research questions that scientists seek to answer.^{3,5,11}

NovaSeq X sequencers empower scientists to increase the scale and scope of their studies with ultra-high-density flow cells and ultra-high-resolution optics. These sequencing solutions make even the most ambitious multiomics projects possible. Experiments that may have been out of budget in the past are now affordable for more researchers, thanks to advanced technologies and more attainable sequencing-intensive multiomics methods. With NovaSeq X, sequencing a genome is roughly one third of the cost of genome sequencing just a year ago. As a result, these systems democratize multiomics, making the cost of multiomics experiments comparable to the previous cost of a single -omic experiment. Along with this advanced NGS technology, the NovaSeq X sequencers are user friendly, with supporting materials, methods guides, and new reagents that enable room temperature shipment without the need for cold chain logistics.^{6,11,12}

Download the NEW Multiomics Methods guide from Illumina to learn how to achieve ambitious multiomics studies.



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Garbage to Guts: The Slow-Churn of Plastic Waste

The earth's oceans contain vortexes of plastic trash. These slow-swirling patches of suspended debris span a collective area of millions of square kilometers.¹ Polyethylene, which is commonly used for medical, cosmetic, food wrapping, and industrial purposes, is the most prevalent marine plastic contaminant.² Much of this waste is microplastic (MP) particles, which disrupt the delicate balance of marine and terrestrial ecosystems

from the tiniest microorganisms all the way up the food chain.

Each week, humans consume up to five grams of MPs³—the equivalent of five paper clips—through various routes, turning the human body into its own reservoir of MP waste.⁴ Researchers discovered MPs flowing through human blood⁵ and islands of MPs in various organs, including the liver, kidney, spleen, lungs, and placenta.^{6,7,8,9,10} Humans also excrete MPs in their stool.¹¹

The gastrointestinal (GI) tract is the most common entryway for MPs, and is a gateway to more widespread systemic dysfunction. In a recent study published in *The Journal of Hazardous Materials*, researchers used an in vitro artificial colon model plus intestinal cell cultures

Humans consume a bewildering quantity of microplastics, which affect the gut's microbiota.

to examine how MP exposure influences the gut microbiota and intestinal barrier.¹²

“Clinical studies are limited by obvious ethical constraints. In vitro human colonic models are valuable tools allowing to maintain a complex and metabolically active human gut microbiota for several weeks under physiologically relevant conditions,” said Muriel Mercier-Bonin, research director at the French National Institute for Agriculture, Food, and Environment (INRAE) and coauthor of the study.

Mercier-Bonin and her team used an adult human colon model—a bioreactor system that mimics the microbi-

ota niches of the human colon—to study how repeated exposure to polyethylene microspheres affects microbial communities and their metabolic activities. Fecal microbiota samples from healthy adult donors were fermented in this model system and exposed to MPs daily for two weeks. Mercier-Bonin and her team examined how the diversity and activity of these microbial communities changed. They also added microbiota samples from the bioreactor to cell cultures of donor intestinal cells obtained from cell banks to study whether MP exposure disrupts the intestinal barrier.

The researchers found that ongoing MP exposure decreased beneficial gut bacteria and increased pathogenic species, along with their production of potentially harmful metabolites associated with gut dysregulation. These metabolites did not significantly disrupt the cultured gut lining, but the healthy adult microbiota samples used in this study may differ from those of individuals with underlying GI disorders, or those from people who are more vulnerable to MP exposure, such as infants.^{13,14} For example, other researchers reported a correlation between inflammatory bowel disease severity and fecal MP concentrations.¹⁵

According to Ian Carroll, a molecular microbiologist and assistant professor in the Department of Nutrition at the University of North Carolina who was not involved with the study, the intesti-

nal microbiota is central to health. Any environmental or lifestyle factor that shifts the balance of the gut microbiota towards pathogenic species can trigger or exacerbate disease. “You have to think about it in the context of disease. Studies like this are great starting points. They’re cross sectional and will tell you what has happened, but what is the consequence? Does [exposure] cause subsequent disease? We don’t know the mechanisms and a lot more research needs to be done,” said Carroll.

As researchers continue to track the ebb and flow of gut microbial communities, the flotsam of marine plastic waste embeds itself further into environmental niches and the food chain. In the end, human GI jetsam may hold the key to unravelling how MP exposure shapes digestive ecosystems and overall health.

—Iris Kulbatski, PhD

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Self Sufficient Cells?

Whenever students approach geneticist Jef Boeke claiming that they’ve made a cool new discovery, his first reaction is always, “Show me the plates.” Boeke is the founding director of the Institute for Systems Genetics at NYU Langone Health, and most of the work in his lab focuses on microorganisms. Looking directly at his students’ cells under the microscope helps him pick out details that could lead to interesting discoveries, so when NYU Grossman School of Medicine PhD student Julie Trolle sent him pictures of her genetically engineered Chinese hamster ovary (CHO) cells in 2017, he knew that he needed to see them for himself. What he saw was the resurrection of an amino acid synthesizing pathway that was lost in animals more than 500 million years ago.

In total, 20 amino acids make up the thousands of different proteins in all life forms. But animals can only make 11 of them; they have to pick up the other essential amino acids from their environ-



Scientists are discovering how microplastics change the diversity and activity of intestinal microbes.

ments. They do this by consuming plants, which typically make all 20 amino acids, or eating animals that have consumed plants. Given that most plants and microorganisms carry the pathway for all 20, the missing nine raise questions about animal evolution: When and why were these pathways lost? And could animals somehow produce the amino acids again? Trolle was particularly interested in using synthetic biology and engineering to address this second question, and in doing so, potentially lay the groundwork for solutions to various medical and industrial problems.

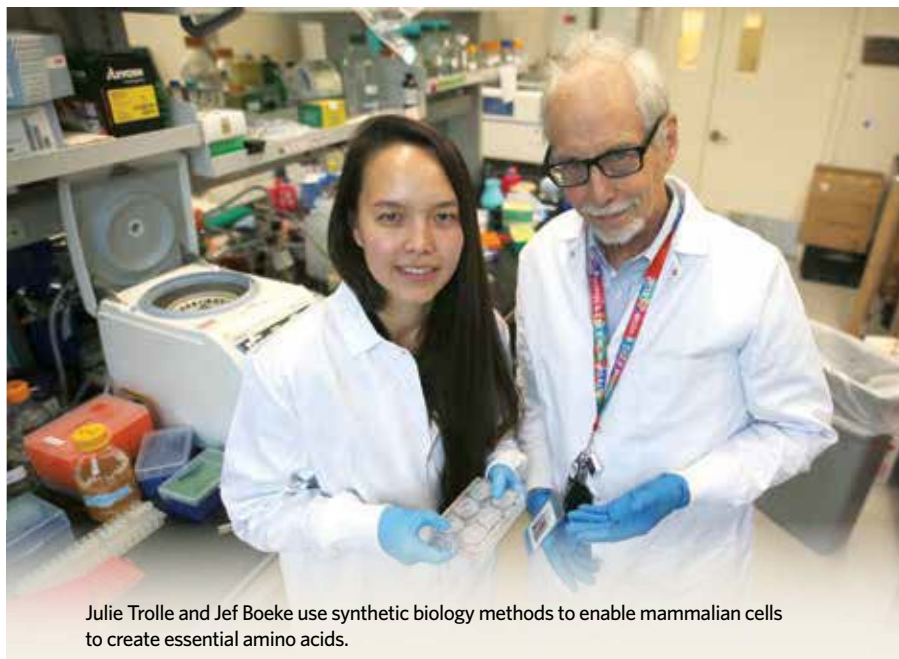
Making the engineered cells didn't come easily, she said. The animal ancestor with these synthesis pathways lived at least 500 million years ago. The interactions between the enzymes necessary to make the amino acids certainly were not the same in those ancestors as in the CHO cells in Trolle's culture plates.

She opted to focus her efforts on valine because of its chemical similarity to isoleucine, another essential amino acid. A cell that could produce valine might also produce isoleucine, she said, potentially making this more efficient than engineering a single amino acid production pathway.

She started by introducing bacterial genes that encode enzymes not normally produced in animal cells into industry standard CHO cells. She hoped that the cells would then be able to convert biochemical compounds in their environment into valine and isoleucine. Trolle put her modified cells and a group of control cells into media that lacked one or both of the amino acids to see whether they'd survive.

To her disappointment, the cells did not survive well without isoleucine in their culture medium, no matter what tweaks she made to the pathway, indicating that they did not make any of the amino acid. However, Trolle's cells continued to survive well in valine-free medium.

The differences in outcome between the regular cells and the genetically modified ones was "more than convincing," Boeke said. The genetically modified cells survived better than control cells, implying that they produced the essential amino acid on their own.



Julie Trolle and Jef Boeke use synthetic biology methods to enable mammalian cells to create essential amino acids.

Trolle measured valine levels in the culture medium to confirm that the cells synthesized valine when fed glucose and pyruvate. "It was an amazing success," said Boeke. "We were so excited." However, when the team submitted the results for publication in 2021, reviewers were skeptical. Valine production in Trolle's cells began to peter out starting at 20 days, and much more so by 40 days. The reviewers noted that the CHO cells ought to be able to produce valine at a consistent rate.

With the help of Boeke and other researchers, Trolle tried different tweaks without success. Finally, however, she made a breakthrough. She discovered that the problem with the pathway was a buildup of an intermediate compound made by the cells. To correct this, Trolle tried adding an extra copy of the gene that coded for the enzyme that converted that intermediate into the next product.

The change worked; suddenly, the cells produced valine at a higher rate than the earlier batch of cells. And valine production increased consistently throughout the 40-day experiment.¹ "It's the first animal cell to produce valine that has ever existed," said Trolle, who is now a postdoctoral fellow in Boeke's lab.

"It's a pioneering and encouraging result," said Andrew Hessel, a cell biologist and geneticist who cofounded the cancer therapeutics company Humane Genomics and cofounded and chairs Genome Project-write, an international research effort focused on genome editing and synthesis. Hessel wasn't involved with the research, although he has previously collaborated with Boeke and another of the study's authors. "This is a really cool first step towards making a fully autotrophic cell," Hessel said.

Now that Trolle has demonstrated that an essential amino acid producing animal cell is possible, Hessel said that it's only a matter of time before others follow in her footsteps with different amino acids. "We will end up seeing a cell that can make all of its amino acids in the next few years," he said.

Trolle hopes that the work will have applications for cell culture methods. The medium used to feed the CHO cells she worked with, for instance, is expensive and often relies on animal products. Working with autotrophic cells would be easier and more cost efficient and would make processes such as growing meat in a lab more feasible. "Any sort of application involving culturing mammalian cells at large scales

is likely going to require some genome engineering in order to make it scalable,” she said. “This is taking a first step towards making that kind of thing happen.”

According to Stanford University synthetic biologist Drew Endy, who was not involved in the study, these pathways could be engineered to create other amino acids that have never before been biosynthesized. Such technology could lead to the production of new proteins and medicines, said Endy, who invests in synthetic biology companies and cofounded and still advises the DNA construction company Gen9.

Moving beyond the CHO cells, Boeke is interested in incorporating the newly engineered pathways into embryonic stem cells, potentially engineering a mouse that could produce its own valine, which might be cheaper to feed in the lab. However, Boeke said that for now, projects such as a fully autotrophic animal cells are still the stuff of science fiction, adding that there may be “some unforeseen consequences of having cells actually produce these [amino acids].”

Hessel is nonetheless impressed by the work, and particularly the use of bacterial genes to enhance mammalian cells. “Our mammalian cells have a lot to learn from bacteria,” he said. “It is remarkable that bacteria are teaching us how to be more independent.”

—Katherine Irving

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Waves of Macro-molecules

Vakil Takhaveev joined Matthias Heinemann’s laboratory at the University of Groningen in 2015 to research cellular metabolism. Early in his studies using budding yeast as a model organism, he peered into the microscope

and observed mesmerizing fluctuations in the autofluorescence of some metabolic markers; he quickly became fascinated by the idea that metabolism oscillates like waves over time. “That was aesthetically attractive to me. I wanted to find out the cause,” he said.

Back then, Takhaveev planned to extend a previous study where data suggested that metabolic oscillations in yeast work in synchrony with cell cycle regulators to drive cell growth and division.¹ Temporal changes in the levels of NADPH and ATP, which play an essential role in many biochemical reactions inside the cell, indicated that certain metabolic pathways are differentially active at specific moments, said Heinemann. The next step was to find out which processes inside the cell created this rhythm.

In a study published in *Nature Metabolism*, Takhaveev and Heinemann reported that different cellular building blocks such as proteins and lipids are not made at the same time during the cell cycle. Additionally, these fluctuations in the production of macromolecules are responsible for the metabolic oscillations they previously described.²

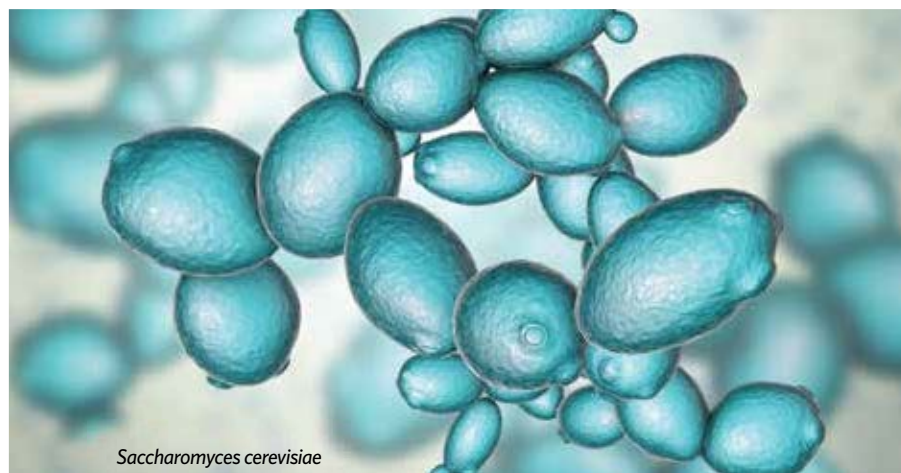
Using the brewer’s yeast *Saccharomyces cerevisiae*, Takhaveev and his colleagues took a closer look at other macromolecules, starting with the production of proteins, a process considered to increase either exponentially or at a constant rate during the yeast cell cycle.

The team engineered yeast to express a fluorescent protein when protein synthesis was active and tracked changes in the fluorescent marker in cells cultured in a microfluidic device that enabled the researchers to visualize single cells over an extended period of time. “We saw two waves, and we were impressed because it contradicted what the field knew,” Takhaveev said.

To validate their protein findings, the team devised a new technique named stop-and-respond, in which they combined their expertise in monitoring the levels of NADPH autofluorescence during the cell cycle with strategies to halt the production of specific macromolecules.

To stop protein synthesis, the researchers used cycloheximide, a well known protein synthesis inhibitor, and looked at the levels of NADPH before and after treatment. “We measured these sensitivities to the inhibitor at different moments of the cell cycle, and found, surprisingly, there are also two waves. If these two orthogonal methods show similar [results], then we concluded that there are two waves of production,” Takhaveev said.

Since they observed an unexpected pattern for protein synthesis, they wondered if other macromolecules such as lipids and carbohydrates also followed a dynamic pattern. Using the stop-and-respond method, they treated cells with a lipid synthesis inhibitor and observed



a peak of NADPH in the second half of the cell cycle. The researchers then perturbed the production of polysaccharides and saw similar fluctuations in production during the cell cycle.

“Biosynthetic processes are partially temporally segregated, meaning that if a cell grows, not all of these processes start to be active,” Heinemann said.

The team next used a mathematical model to predict how these waves would influence metabolic rates. “There are changes in primary metabolism to satisfy these changing demands in macromolecule production,” Takhaveev said.

These patterns of macromolecule production timing during the cell cycle are relevant contributions, said Nicolas Buchler, a biophysicist at the North Carolina State University who was not involved in the study. “[Scientists have known] from yeast that when the bud is formed, there is a massive growth in volume, which is going to be lipids and carbohydrates,” he said. “It is happening right where you would expect it.”

Michael Polymenis, a biochemist at Texas A&T University who was also not involved in the study, said that the findings are an important first step as they show that global biosynthetic processes are dynamic. “Traditionally, [metabolism] has been seen as something that just goes on and on in the background, as opposed to cell division, which has very specific landmarks. Now we know, if this is corroborated by additional studies, that we have landmarks in cell growth—dynamic landmarks. Hopefully, we will find specific pathways that talk to each other,” Polymenis said.

—Mariella Bodemeier Loayza
Careaga, PhD

The Fish Loggers

Every morning just before dawn, Eric Bricston launches his fleet from the shores of La Playita, a tiny beachside town on the Western coast of the Baja peninsula. After pushing their boats directly from the bone-white sandy shores, he and his fellow fishermen head toward Gordo Banks, a famous fishing spot known for its enormous pelagic fish such as wahoo tuna (*Acanthocybium solandri*), mahi-mahi (*Coryphaena hippurus*), blue marlin (*Makaira nigricans*), and other catches that weigh hundreds of pounds each.

Once a construction worker living in Utah, Bricston moved to Mexico in the 1980s. He established his fleet shortly after and now manages five dozen pangas—open-top, fiberglass fishing boats used throughout Mexico and Central and South America. Local people have been fishing in La Playita for generations. Now, it’s a popular tourist destination, especially for fishing—even as the number caught each year declines.

Throughout his time there, Bricston has been recording the catch his fleet brings back. In the beginning, he noted everything he fished each day, but that was a lot of effort, he said, so he switched to weekly fish reports. In 2010, he began publishing the data online.

Bricston intended his reports for fishermen to use so they could know which species were visiting the bay in a given week and what they might expect to catch. But in 2011, the logs caught the eye of William Gilly, a Stanford University marine biologist who studies the effects of climate change in the Gulf of California. Shortly after the 2009–2010 El Niño event, Gilly had begun a study on how and why Humboldt squid (*Dosidicus gigas*), normally abundant throughout the Pacific coast of Mexico and one of the main species caught commercially, had suddenly almost vanished from the region.¹ While trying to hunt down information on the squid, Gilly came across Bricston’s logs online.

Curious about whether Bricston’s fleet had caught any squid in Gordo Banks, he

got in touch, and the pair began emailing back and forth. They finally met in person at a taqueria in Cabo San Lucas in late December 2011 when Gilly was in the region. The conversation led to a collaboration that spanned the following decade, providing novel insights into ecosystem changes underway in the region.

“[Bricston] was super dedicated at keeping records,” said Gilly. And although he didn’t document the size of the squid he caught, something that Gilly’s team monitored separately through their own field experiments, Bricston provided Gilly with the numbers of fish and squid that his fleet had caught since the 1980s.

It was an ideal resource to complement Gilly’s investigation into the mystery of the disappearing squid. After the El Niño event, various data showed that the squid slowly returned to the region, but they were a smaller size than they had been previously. Gilly had an idea of why this might be.

Humboldt squid can have two phenotypes as adults, large and small, which depend on the water temperatures where they mature. “El Niño makes the water at depth unusually warm,” explained Gilly. Previous studies had revealed that during and following previous strong El Niño events such as one in 1996, average squid body size had dropped briefly—that is, there were more of the small phenotype—but returned back to normal after a few years.

After the 2010 event, however, the large phenotype failed to return at all. There was another El Niño warming event in 2015. “The squid just switched to this small phenotype. And they’ve been there ever since,” Gilly said.

In addition to direct effects on squid development of subsurface water temperatures, which increased year-on-year from 2010 to 2016, it’s possible that other factors contribute to the continued dominance of this small phenotype, such as a decrease in the abundance of sardines and other small pelagic fish that the squid prey on, Gilly and colleagues suggested in a recent paper describing their research.² Sardines are sensitive to temperature, and their numbers also dropped dramatically in 2010.³ Since these two warming events, the squid fishing industry, which

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The sailfish (*Istiophorus spp.*) chases a group of fish that have been lured by a bait trap.



relies partly on large-size catch to remain commercially viable, has collapsed. Even though subsurface water temperatures have cooled slightly since 2016, large squid still haven't returned to the region, perhaps reflecting an irreversible ecological shift in the region.

Gilly also started to piece together another story from Bricston's data and from logs by another fisherman in the area, a former chemist and hobbyist named John Snow who looks for rare fish in the Los Cabos region. Snow has documented the fish he's caught since the early 2000s, and also keeps daily personal logs, said Gilly, who first reached out to Snow in 2011. As panga fishermen often fishing in the same area, Bricston and Snow knew each other after meeting in 2003.

A couple of years ago, Gilly gathered all of the data Bricston and Snow had on the number of fish of each species they had caught between 2003 and 2020. From Bricston's data on more than 250,000 fish, Gilly quickly gathered that for "all sport-harvested species, the numbers caught per trip had gone down consistently over the last 15 years." Bricston has observed such trends, and there are some species that he doesn't

see at all anymore. Snow's smaller but more diverse dataset corroborated the conclusion.

According to Gilly, the sport fishermen of Los Cabos blame the decrease on overfishing from commercial boats, which make up a billion-dollar industry in Mexico. But the true story is more complicated, he added. For one thing, migratory species typically aren't as affected by overfishing as are local species, yet both sorts of fish are declining. What's more, over the past 15 years, "the fishing pressure doesn't seem to have gone up" based on commercial and sport fishing logs, Gilly said. Nevertheless, the cause of the decrease remains hard to untangle for pelagic fish. Multiple factors may be at play simultaneously, said Gilly, who included the fish data in his group's 2022 squid paper. "It's a huge mess."

"I thought [the study] was great," said Andrew Leising, a research oceanographer at the Southwest Fisheries Science Center, part of the National Oceanic and Atmospheric Administration, who was not involved in the work. "It shows something we've been thinking about for a long time, which is how the changes in the temperature of the deeper water is going to affect animals, particularly squid." He added that

the situation may be more complex for other marine species, noting that the effects of climate and other factors on many migratory fish are hard to tease out because these species experience so many different environments and fishing pressures as they travel.

What's clear to Gilly and other scientists, though, is how much research like this benefits from data collected by citizen scientists. Phil Hastings, curator emeritus at the Scripps Institution of Oceanography, said that Snow has donated more than 100 specimens to the institution's collection, some of which were previously unidentified. Snow also provides photographs and descriptions to Ross Robertson, a marine biologist at the Smithsonian Tropical Research Institute in Panama who runs and maintains multiple websites documenting the fish in the greater Caribbean and the Tropical Eastern Pacific. Such data provide "information on patterns of diversity," Robertson said. "And that's useful for conservation and management."

Since the pandemic, Bricston has stopped keeping fishing logs since the process is time-consuming. He's also close to retirement and is in the process of downsizing now that his sons have left home. Gilly said that there should likely be a permanent position counting and logging fish in Los Cabos, but that so far, there's no such role. "It's a really unique and valuable dataset," Gilly said of Bricston's fish reports, "It's really worth doing because it's a good independent source of what's really going on."

—Natalia Mesa, PhD

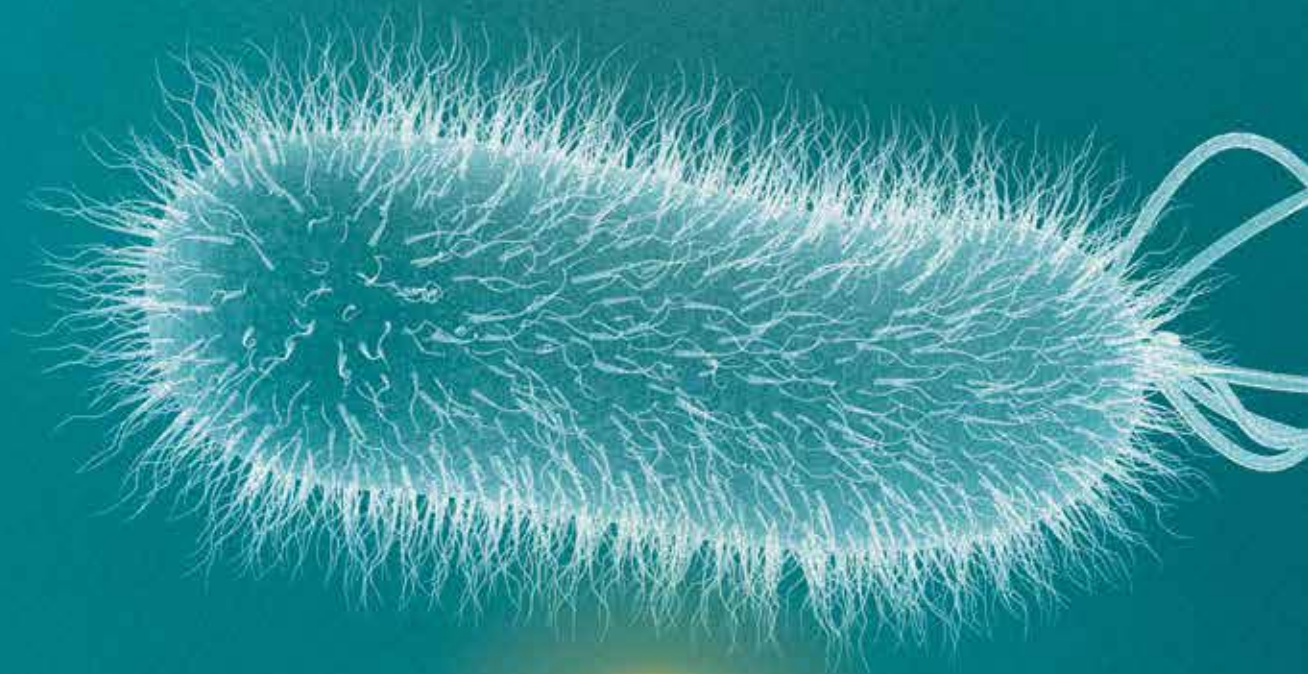
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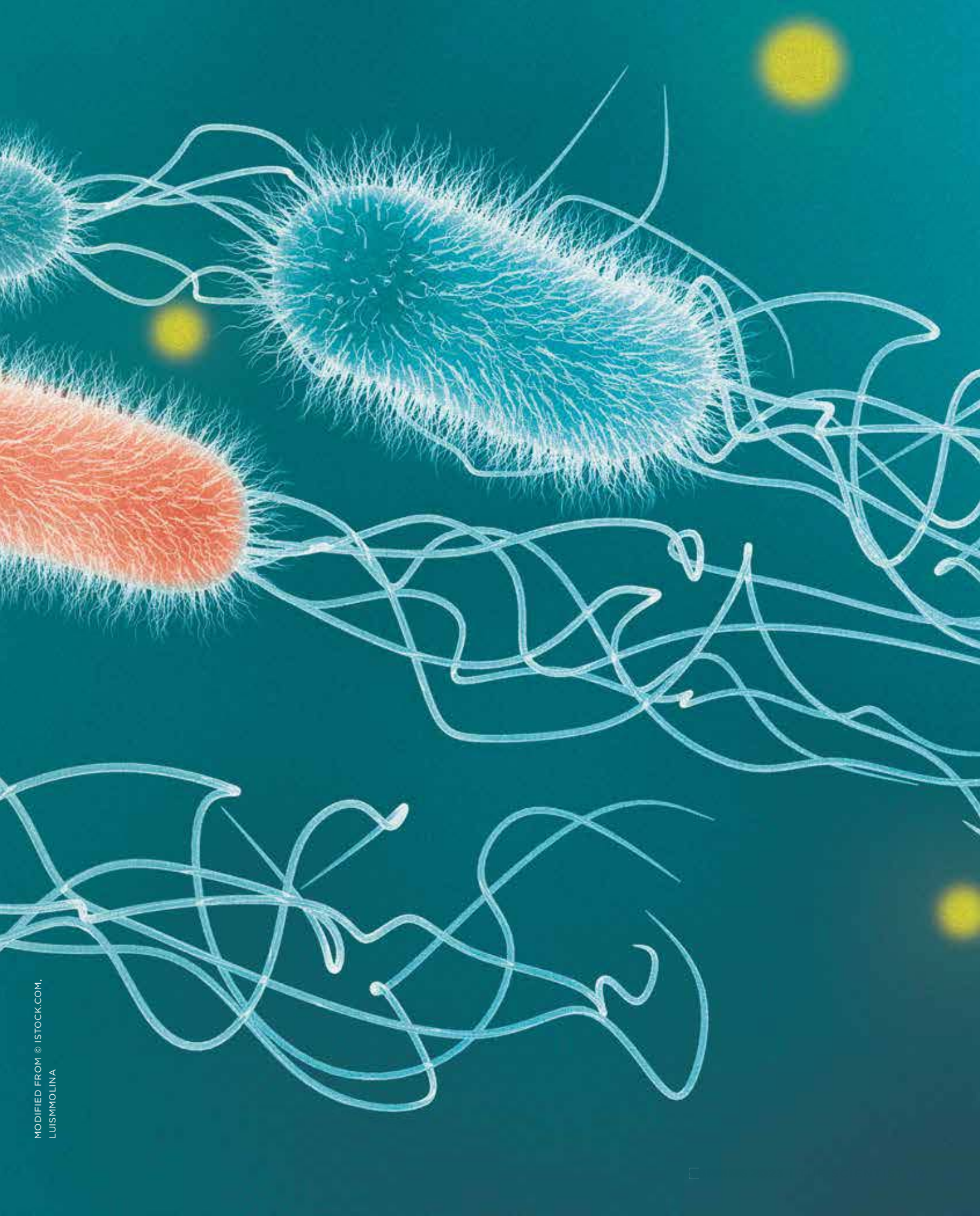
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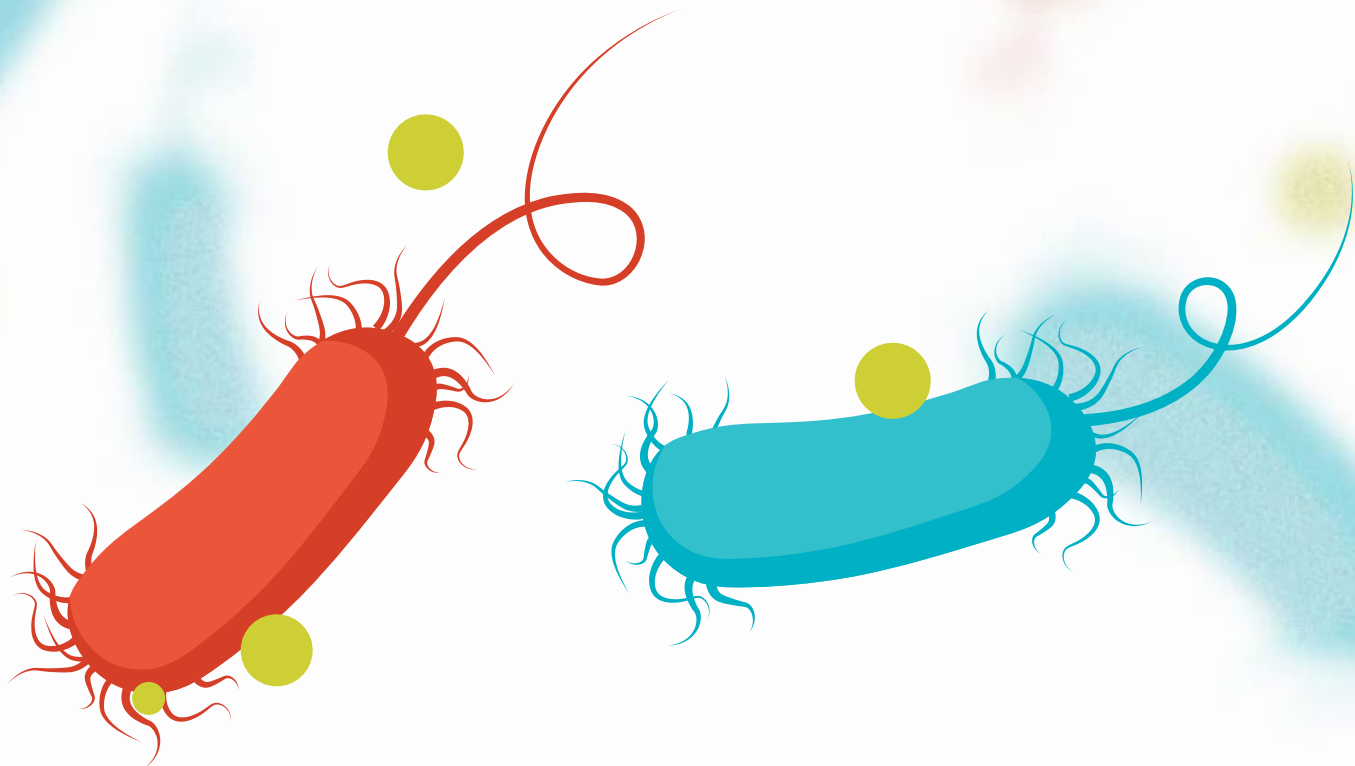
Cooperation and *Cheating*

Bacteria cooperate to benefit the collective, but cheaters can rig the system. How is the balance maintained?

BY MARIELLA BODEMEIER LOAYZA CAREAGA, PhD







People often recognize social behaviors in complex organisms such as insects, nonhuman primates, and humans. But Megan Frederickson, an ecologist and evolutionary biologist at the University of Toronto, is interested in a different, microscopic social community: bacteria. “Cooperation is everywhere,” she said. “Cells cooperate in multicellular organisms; individuals cooperate in societies; and different species cooperate... Why would it not be the case that microbes cooperate with each other?”

Researchers have known for more than 20 years that bacteria participate in collective behaviors such as forming biofilms and acquiring nutrients from the environment. But being part of a cooperative group does not necessarily mean that every individual bacterium plays by the rules. Occasionally, cheaters arise.

Conflict in bacterial communities

For cheating to happen, it must arise from an adaptive strategy, said Frederickson. “[The individual] should have a fitness benefit from not cooperating or not cooperating as much as others in its environment.” Additionally, for a bacterium to be considered a cheater, it has to evolve from an ancestrally cooperative state. “We should reserve the word cheating to mean a breakdown in cooperation,” she added.

Cheaters exploit the work of others by consuming shareable resources that are costly to produce for an individual but benefit the entire population.¹ These public goods can take many forms in a bacterial community: exopolysaccharides to build biofilms, enzymes that degrade antibiotics, or scavenging molecules to cope with nutrient scarcity in the environment.²

But how does a bacterium become a cheater? Unlike humans, bacteria do not make an intentional decision to cheat. “[These individuals] lack a gene or have a protein that is no longer functional. That is why they stop making a social trait and become cheaters,” said Rolf Kümmerli, an evolutionary biologist at the University of Zurich who studies how bacteria share metabolites such as enzymes and iron-scavenging molecules.

We should reserve the word cheating to mean a breakdown in cooperation.

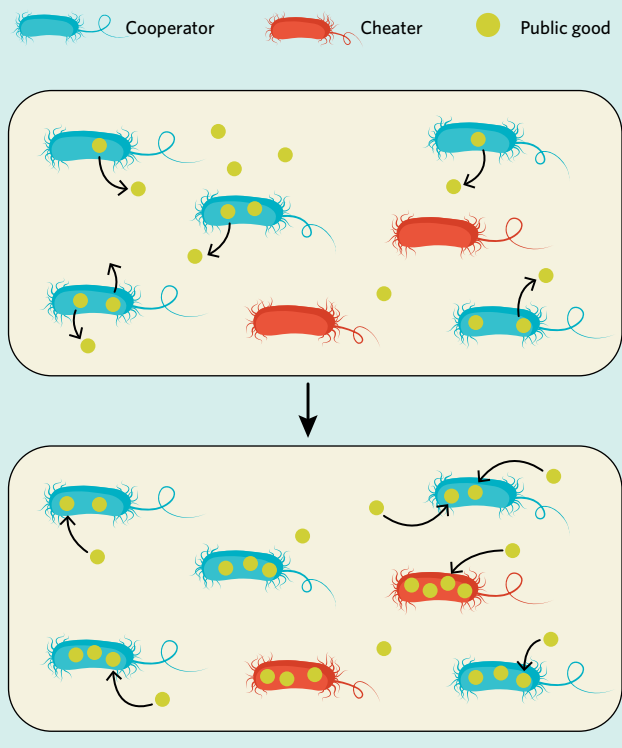
—Megan Frederickson, University of Toronto

Armed with this knowledge, researchers tweak bacterial genetic material to better understand cheating. For instance, they can knock out genes responsible for the production of shared metabolites to generate cheating mutants in the lab. Then they can culture these nonproducers along with producers and measure their relative fitness, that is, the ratio of the growth rates of the two strains. If a resource is essential for growth, nonproducers should outgrow producers in a medium containing both strains as they consume the shared resource without paying the metabolic cost to produce it.

This bacterial arms race appears for many communal compounds. For example, by using the opportunistic pathogen *Pseudomonas aeruginosa*, scientists showed that cheaters can exploit iron-scavenging compounds called siderophores,³ as well as pro-

CHEATERS BENEFIT FROM COOPERATORS IN BACTERIAL COMMUNITIES

In cooperative bacterial communities, cheaters may arise. These bacteria do not pay the cost of producing public goods but still consume the shareable resources produced by other cooperative bacteria.



teases used to digest nutrients.^{4,5} Another example of an exploitable compound is a bacterial enzyme used to degrade antibiotics. Researchers conducted experiments using *E. coli*, which resist β -lactam antibiotics by producing β -lactamase enzymes. Mutants that do not produce those enzymes tolerate higher doses of ampicillin, a β -lactam antibiotic, for a longer time when sharing a biofilm with enzyme producers.⁶

An intriguing question for many scientists is what makes bacterial cooperation persist when cheaters exist in several natural and disease settings.² “It is really fascinating why we do not see [cheating] happen more often. We see lots of examples of cooperation in bacteria and, by and large, they are not undermined all that often by the evolution of [cheaters],” said Frederickson.

Keeping cheaters at bay

One idea that partially explains cooperation is frequency-dependent selection. According to this idea, a cheater geno-

type appears when there are few cheaters among many cooperators. If the cheater genotype becomes more frequent, the proportion of producers might decrease to a point that being a cheater is no longer an adaptive strategy, causing this genotype to be selected against.

Additionally, cooperators use different strategies to optimize public goods usage and reduce cheaters’ access to them. One such approach is spatial structuring, that is, the 3D arrangement of a bacterial community.

“Spatial structure is the easiest answer for why we don’t have cheaters running wild,” said Steve Diggle, a molecular microbiologist at the Georgia Institute of Technology who investigates microbial interactions and social behaviors. In the real world, bacteria are commonly organized in densely packed biofilms—slimy, multicellular structures—that favor cooperation. Biofilms help cooperators by keeping related individuals physically close to each other and limiting the diffusion of shared compounds due to its viscous composition. Kümmerli’s group replicated a biofilm environment in laboratory experiments to confirm this notion. The team cultured *P. aeruginosa* siderophore producers and nonproducer mutants in a viscous growth medium and found that limited dispersal of the iron-loaded scavenging molecules resulted in increased fitness of the producer population.⁷

Spatial structuring also helps bacteria keep their kin closer, and relatedness favors cooperation. “Relatedness is obviously a really key driver of social interaction. So, generally, the higher the relatedness between interacting partners, the more cooperation will be selected for because those cooperative genes are shared between the cooperator and the recipient,” said Siobhán O’Brien, an evolutionary ecologist at Trinity College, Dublin.

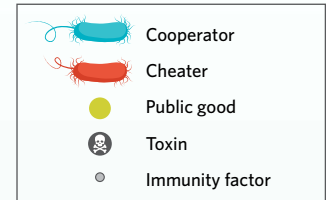
This idea, called kin selection, explains why a trait that does not directly benefit an individual might be favored in a bacterial group where a bacterium is most likely surrounded by cells sharing a similar genetic background. By helping a neighboring cell do well, a bacterium ensures that its genes pass on to the next generation.

These individuals lack a gene or have a protein that is no longer functional. That is why they stop making a social trait and become cheaters.

—Rolf Kümmerli, University of Zurich

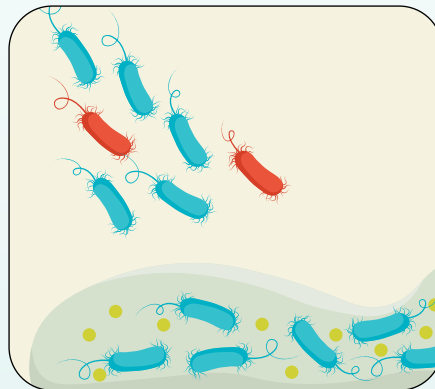
Another approach by cooperators to minimize cheater populations is to direct communal resources only to their kin. “Some public goods...are quite specific. They can only be taken up by cells with the correct receptor, and normally that means they are

STRATEGIES USED BY COOPERATORS TO CURB THE CHEATER POPULATION IN A BACTERIAL COMMUNITY



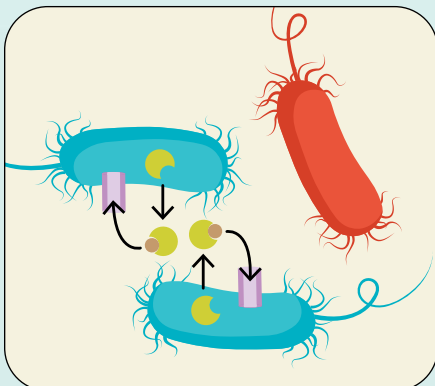
SPATIAL STRUCTURING AND KIN SELECTION

As cooperators build biofilms, they keep their kin close and limit cheaters' access to communal resources.



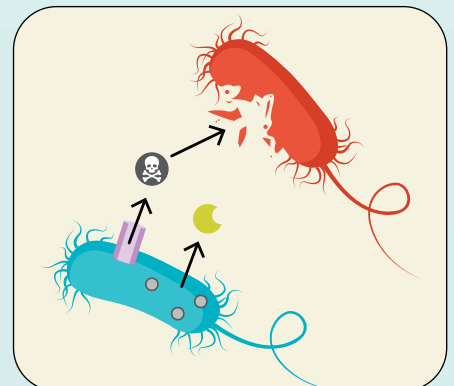
KIN DISCRIMINATION

Cooperators discriminate kin from non-kin and share resources only with highly related cells.



POLICING

Cooperating bacteria can sanction cheaters by producing diffusible toxins together with a resource.



kind of strain specific. If [there is] another strain of the same species that is quite distantly related, they won't possess the correct receptor to take up the public good," O'Brien said. The iron-scavenging molecule pyoverdine is a good example of this. In a natural community, researchers showed that some pyoverdine producers secrete incompatible pyoverdines that cannot be used by nonproducers, suggesting that these cheaters lack the receptors needed to take up these molecules.⁸

We think of bacteria as simple organisms, but they are not. They have been evolving on this planet for a very, very long time. And they are capable of some very sophisticated things. I think we don't give them quite enough credit.

—Megan Frederickson, University of Toronto

Discriminating kin from non-kin also enables cooperators to directly sanction cheaters by policing them. To police communities, cooperators may produce an energetically costly compound, often a toxin, to control the number of cheaters within the group. For instance, *P. aeruginosa* produces a shareable protease along with a toxin, cyanide. Cooperators produce an immunity factor that protects them from the cyanide, thus selectively killing cheaters.⁹

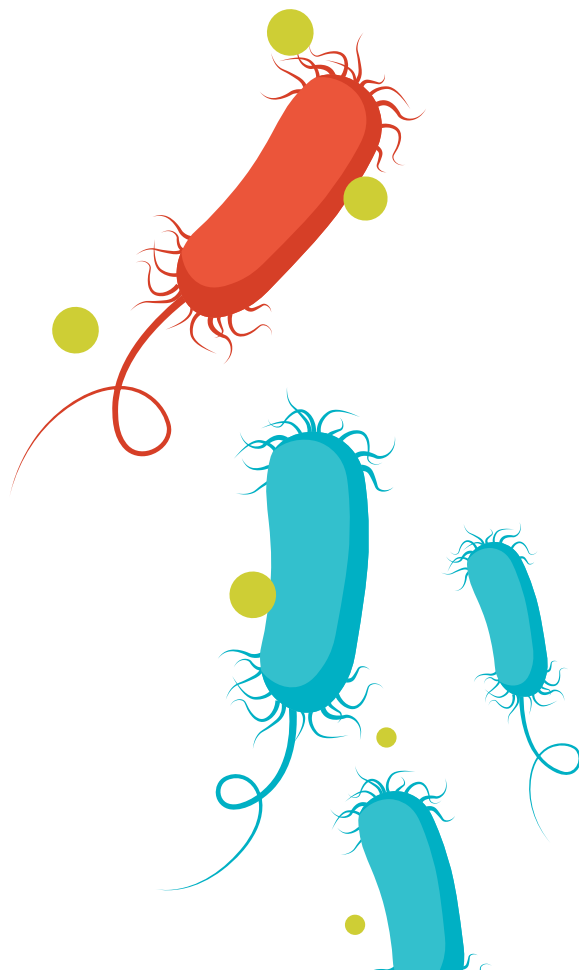
As social creatures, bacteria perform a variety of collective behaviors that enable them to thrive in different environments. Like other social animals, they must also deal with individuals that do not cooperate but still want to take a share of the goods produced by others. Although cheaters threaten bacterial cooperation, they do not undermine it as both cooperators and natural selection weigh in to control their spread.

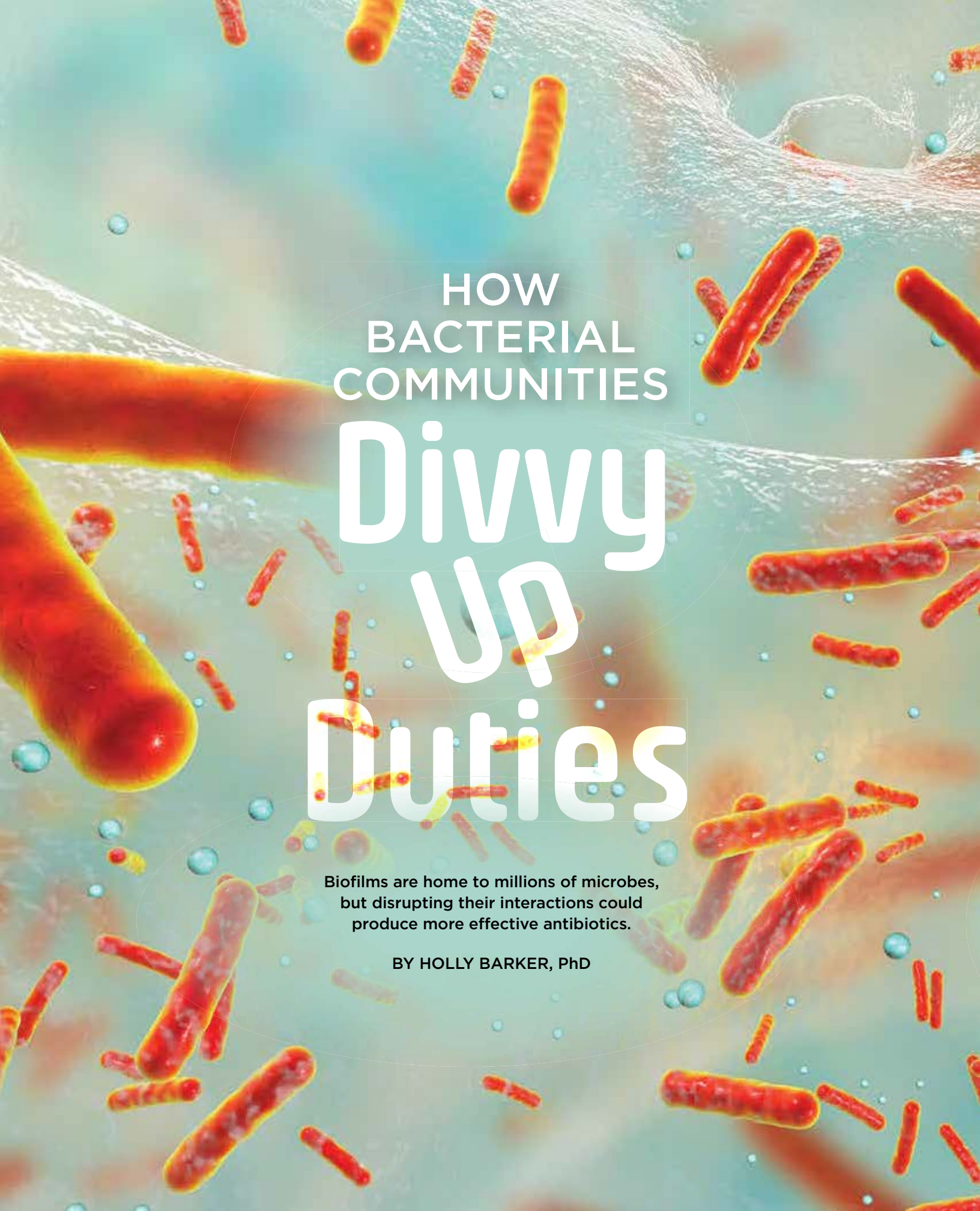
"The interest in the social lives of microbes is because they make a good experimental system for our larger ideas about how cooperation works, including in animals or in plants," Frederickson said.

Additionally, a better understanding of the social dynamics of bacteria might help us either come up with new therapies or offer ideas that we could use for biological control, added Frederickson. "We think of [bacteria] as simple organisms, but they are not. They have been evolving on this planet for a very, very long time. And they are capable of some very sophisticated things. I think we don't give them quite enough credit." ■

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HOW BACTERIAL COMMUNITIES Divvy Up Duties

Biofilms are home to millions of microbes, but disrupting their interactions could produce more effective antibiotics.

BY HOLLY BARKER, PhD



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Sitting on her dirt throne and swollen with eggs, a termite queen is tended to by her sterile subjects. All of the insects in the colony are specialists in what they do: Workers forage and construct the mound, while soldiers defend it from invaders. Division of labor among social insects maximizes efficiency to ensure that the royal genes make it to the next generation. Yet, teeming in the soil around them, a similar scenario plays out in a whole other domain of life.

The evolution of bacteria has a lot in common with higher eukaryotes such as birds and bees, said Ákos Kovács, a microbiologist at the Technical University of Denmark. Studying single-celled organisms may help scientists better understand multicellular life, he added.

Kovács's team investigates "sociomicrobiology," an emerging field that examines the rich social lives of microbes. In contrast to the free-living, or planktonic, bacteria commonly studied in the lab, most prokaryotes in nature settle down in complex communities, often consisting of several interacting species. These biofilms of aggregating microbes are everywhere: on rocks, bathtubs, and medical devices. In fact, you're supporting a heap of these communities right now. The slimy coating on unbrushed teeth is biofilm, and the beneficial microbes on your skin and gut often grow collectively.

Biofilms form when groups of bacteria cover themselves in a sticky mixture of sugars, protein, and DNA. This extracellular matrix glues bacteria to surfaces and serves as a slimy shield, protecting cells in the interior from predators and antibiotics. Conventional drugs become ensnared in the matrix, tangled in a molecular mesh that prevents them from penetrating the bio-

film's inner layers. What's more, oxygen depletion causes cells in the center to enter a hibernation-like state, making them tolerant to antibiotics that target metabolic processes. In fact, biofilms can withstand doses up to 1,000 times greater than their planktonic counterparts.

Within the biofilm, bacteria take on different responsibilities. Some individuals focus on reproduction to expand the colony, while others specialize in construction, oozing polysaccharides and proteins that make up the extracellular matrix. And other bacteria defend the community, building molecular weapons that shoot competitors with toxins.

These tasks aren't fixed for life. As the biofilm matures and the community needs change, an individual bacterium may take on new responsibilities. Kovács's team has found that in populations of the soil bacterium *Bacillus subtilis*, most microbes assume responsibility for matrix production during early development when they are little more than a throng of unconnected cells. But once construction progresses, some cells will switch to producing spores or useful enzymes.¹

A microbial metropolis

So how do bacteria dole out chores? It's partly stochastic, said Kovács. By amplifying random fluctuations in cellular reactions, individual bacteria specialize into distinct roles. For instance, the cells within a *B. subtilis* biofilm that are engaged in protease production are randomly determined.

Task delegation can also be influenced by a bacterium's location within the biofilm, said Daniel Dar, a researcher of microbial systems at the Weizmann Institute of Sciences. Just as different boroughs of a city might differ in levels of air pollution or abundance of organic supermarkets, different parts of the biofilm are exposed to wildly different levels of oxygen and nutrients. Bacteria sense these microenvironments and adapt by up or down regulating the expression of certain genes to influence different cell processes.

As a postdoctoral researcher in Dianne Newman's lab at the California Institute of Technology, Dar developed a way to track gene activity across the entire biofilm with submicrometer resolution. He used a tool known as parallel sequential fluorescence in situ hybridization (par-seqFISH) on populations of *Pseudomonas aeruginosa*, a species often found in the lungs of people with cystic fibrosis.

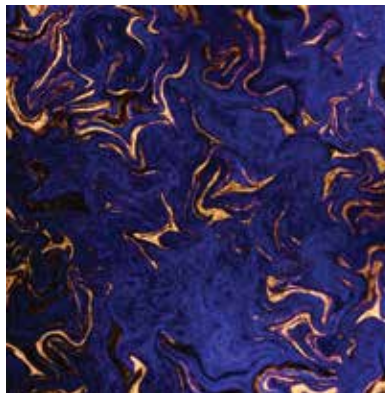
Rather than detecting each gene with a single fluorophore as with standard FISH, sequential FISH targets multiple probes to different regions of a transcript, then decodes its identity based on the color sequence. Dar performed the technique on several stages of bacterial growth—from planktonic to mature biofilms—in a single experiment.

By lighting up expression of more than 100 genes, the researchers uncovered what looked like a color-by-numbers map of zoning regulations. Different clusters of bacteria showed diverse patterns of gene activity corresponding to altered metabolic states depending on their locations within the biofilm. For instance, microbes in the bottom portion of the mature biofilm had activated a set of genes that code for

digestive enzymes, while their westerly neighbors prioritized transcription of genes involved in defense.²

One such defense mechanism was the enhanced production of pyocin, a toxin that affects other bacterial species, by *Pseudomonas aeruginosa* as they begin to aggregate. As the biofilm forms, clusters of cells synthesize pyocin and release it by annihilating themselves. The bacteria burst, sprinkling the poison and DNA into their surroundings. This seemingly suicidal behavior gives the biofilm the best chance of survival: The toxin kills competitors, while the DNA binds to molecules within the matrix, strengthening its structure and accelerating biofilm growth.

While the tool could provide new leads for treating biofilm infections, researchers also need to consider environmental factors. "It's difficult to understand behavior without understanding context," said Newman, who teams up with chemists to visualize metabolite levels. One way she hopes to do this is by tagging oxygen molecules to illuminate the gradients between the oxygenated extremities and the anoxic inner core. Combined with spatial transcriptomic techniques and other methods, the approach could allow researchers to learn how conditions within the biofilm determine bacterial behavior.



This is a false colored mesoscopic image of two strains of *E. coli* expressing different fluorescent proteins. As the populations expand, they clash with adjacent colonies and produce a marbling effect running through the biofilm.

Chromosomal contortions

Division of labor doesn't only arise from differences in gene expression but can also be triggered by irreversible genetic changes. Among *Streptomyces coelicolor*, a soil-dwelling bacteria that forms fungal-like structures, deletions of large chunks of their chromosome causes a subset of cells to specialize in antibiotic synthesis. More than half of the antibiotics used to treat human infections are produced by *S. coelicolor*.³

Although the colonies start out as clones, unstable DNA regions quickly acquire mutations. In a paper published this year in *Molecular Systems Biology*, scientists reported how the *S. coelicolor* genome is split into two main sections. The more stable end of the chromosome houses genes that synthesize antibiotics, while the growth-promoting genes are located on the more fragile end. Mutations in these delicate regions often erase sequences

They were killing each other like crazy.

—Bonnie Bassler, Princeton University

associated with growth and replication, generating strains that are specialized in antibiotic secretion.⁴

Specialization comes at a cost, however. Like sterile worker insects, antibiotic producers are unlikely to reproduce, yet the whole colony benefits from their labor. In some cases, mutants were up to 10,000 times less likely to release spores.

Other work revealed how genetic diversity can have more insidious causes. In a paper published last year in *Cell*, researchers described how microbes shoot their compatriots with toxins to drive the emergence of useful mutations.⁵

To defend themselves from competitors, Gram-negative bacteria assemble a molecular machine gun called the type VI secretion system (T6SS).

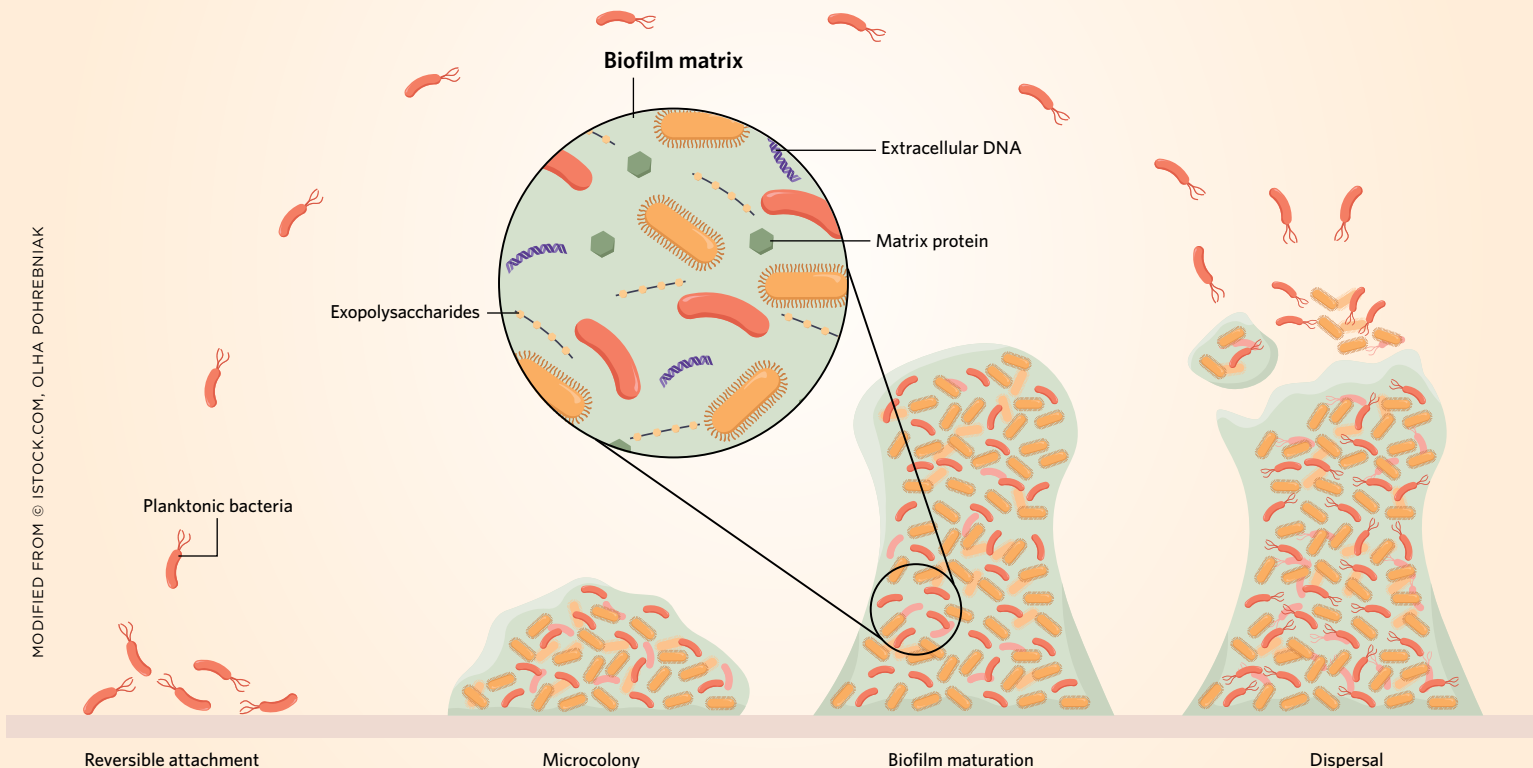
Shaped like a spear, the weapon is loaded with toxins that are fired into adversaries by puncturing their cell walls. To avoid poisoning themselves, the bacteria synthesize immunity proteins that neutralize the toxin.

Scientists have known for decades that bacteria use the T6SS to snuff out their non-kin neighbors, or strangers that are invading their territory. But no one imagined that it's used to kill their siblings, said Bonnie Bassler, a Princeton University molecular biologist and an early pioneer of research on bacterial communication.

The study explained something that researchers call sectoring, which occurs when a biofilm partitions into zones made up of genetic variants, despite the bacteria starting out as clones. Bassler's team grew biofilms made up of the cholera-causing bacteria *Vibrio cholerae*. Just before the community segregated into mutants, cells within the biofilm started shooting their neighbors with toxins. "They were killing each other like crazy," said Bassler.

THE BIOFILM LIFE CYCLE

Bacterial communities form when free swimming bacteria reversibly attach to a surface. At high cell densities, many species trigger processes involved in biofilm formation, including the production of extracellular matrix. The matrix adheres the community to the surface and provides protection from predators and antibiotics. As the biofilm matures, bacteria specialize to perform different tasks within the biofilm. For instance, a subset of cells sprouts flagella and swims off from the community to colonize new surfaces.



To save energy, bacteria only start to assemble their molecular weapons when there's plenty of other microbes around. It's controlled by a process called quorum sensing, a form of bacterial communication. Each bacterium leaks a chemical trickle, which increases in concentration as the population grows. Once a threshold is reached, bacteria activate quorum-sensing genes in unison, triggering group behaviors such as biofilm formation.

The microbial massacre may drive the emergence of quorum-sensing mutants that can resist killing, such as variants that synthesize more of the polysaccharide that can serve as a protective armor. Some of the mutations that Bassler's team spotted in *V. cholerae* have also been found in clinical samples, suggesting that kin killing could be responsible. Murdering your siblings might be a way of speeding up evolution, said Bassler. Bacteria are mutating in all sorts of ways, but "only mutants that can resist T6SS killing survive and replicate, so they flourish," she added.

Worker's strike

By understanding how division of labor is controlled, researchers might be able to disrupt this process to spark societal collapse. Initial findings have pointed to electricity to do just that.

Electricity can kill bacteria, but lower doses can also stimulate growth of specific cell types, said Gürol Süel, a biophysicist at the

University of California, San Diego. Over the past decade, Süel's team has uncovered how bacteria communicate through electrical impulses, much like a giant action potential that passes through the entire biofilm. Electrical signaling allows cells within the biofilm to regulate growth, share nutrients and recruit outsiders to join the community.

In his new work, Süel's team focused on two major biofilm cell types: motile cells, which swim off and colonize new surfaces, and matrix-secreting cells, which churn out the sticky polymers that glue the rest of the colony together.

The team developed a device to electrically stimulate a growing *B. subtilis* biofilm. Despite being genetically identical and exposed to the same microenvironment, the weak electric shock promoted growth of motile cells but not matrix producers. Electrical stimulation skews the ratio of cell types within the community, which is soon overrun with swimmers.⁶

This could lead to biofilm dispersal since less matrix is produced to hold the community together, the researchers suggested. Since the matrix also blocks antibiotics from the biofilm's core, it could make them more vulnerable to treatment, they added. Dispersing bacterial communities by switching their responsibilities is something that Süel's group is currently pursuing.

KILLING THE CORE

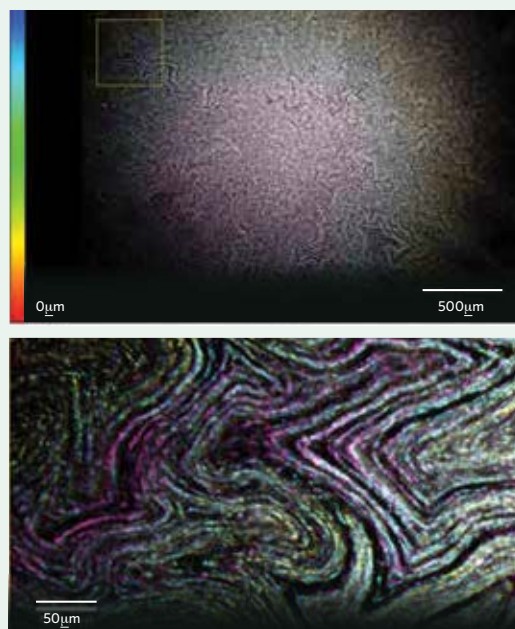
Spatial transcriptomics could provide clues on how to sneak antibiotics into the biofilm's impenetrable core by identifying proteins essential for biofilm formation. Dar's par-seqFISH analysis pointed to a group of metabolites known as phenazines, which are expressed as the *P. aeruginosa* begin to clump together. The finding supports previous data from Dianne Newman's group on how pyocyanin, a type of phenazine, encouraged bacterial cells to interact. Destroying pyocyanin using the enzyme PodA made biofilms more vulnerable to antibiotics, but PodA is unstable and synthesized naturally in minute quantities.⁹

Newman teamed up with Sarel Fleishman to figure out a way to tweak PodA's structure. A structural biologist at the Weizmann Institute of Sciences, Fleishman used a computational approach to predict mutations that increase the protein's stability. When Newman's group applied the customized enzyme to populations of *P. aeruginosa*, the antibiotic destroyed a larger proportion of the biofilm than did the drug on its own.¹⁰

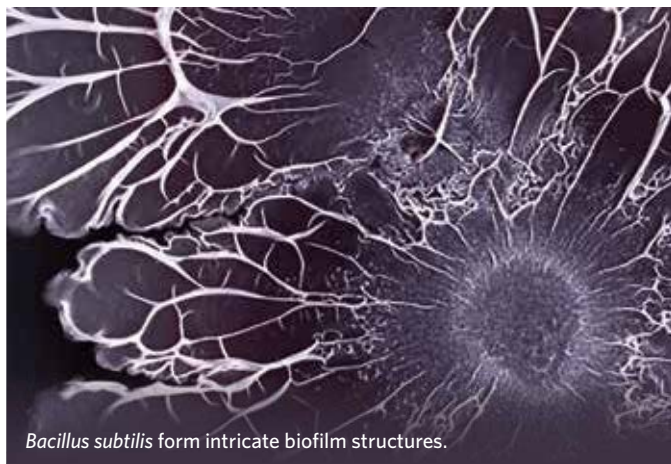
Advances in optical microscopy have provided other clues to weakening the biofilm's core. By redesigning the objective lens, Gail McConnell, a physicist at the University of Strathclyde in the United Kingdom developed a tool to image embryos with high resolution and magnification in a single shot. When Liam Rooney joined McConnell's lab, he was the first microbiologist to get his hands on it and to glimpse the inner workings of a microbial city.

Before Rooney began the project, he had never seen a high-resolution image of the interior of a biofilm. "We assumed it was just a ball of homogenous cells, all densely packed with no real internal structure," said Rooney.

By applying the tool known as the Mesolens to *Escherichia coli* biofilms, the team observed a network of microscopic water channels meandering through the colony's interior.¹¹ These conduits may help the biofilm traffic nutrients, oxygen, and other resources into the starving core, just as our blood vessels ferry molecules around our body. The channels could be exploited to maneuver antibiotics into the heart of the biofilm, said Rooney.



E. coli biofilms observed under a Mesolens reveal channel structures permeating throughout the colony.



Bacillus subtilis form intricate biofilm structures.

Another promising strategy is to target the shared substances, digestive enzymes, matrix polymers and secretion systems, that allow microbial societies to thrive, said Kovacs. When conventional antibiotics are applied to biofilms, resistant cells soon have the upper hand and take over the population. But when bacteria are exposed to biofilm-dispersing drugs that inhibit production of “public goods,” resistant cells are at the mercy of their nonresistant counterparts. The new resources are shared and exploited by surrounding cells, which benefit without expending any energy. The nonresistant cells prevail, growing until the public good runs out and the biofilm disperses.

Testing this approach experimentally, microbiologist Hans Steenackers and colleagues at KU Leuven in Belgium grew *Salmonella* incapable of synthesizing matrix polymers. Unlike wild type microbes, the mutant bacteria were less likely to form biofilms and more vulnerable to antibiotics. No cells evolved resistance over the 40-day experiment, while resistance emerged against standard antibiotics in a matter of days. When the team hunted down some resistant strains and added them to the Petri dish, the nonresistant cells quickly outcompeted their neighbors.⁷

Interfering with public behaviors has potential beyond microbiology, Steenackers wrote in a review on the topic.⁸ The strategy could be extended to treating cancer, which grows in a matrix-like microenvironment, or to disperse insect swarms. Insects communicate via pheromones in a way similar to how bacteria communicate via quorum sensing, said Steenackers, and disrupting cooperation may be a more sustainable approach than typical insecticides.

Studying bacterial cooperation is not only key for targeting pathogenic biofilms, but it could also shed light on multicellular life, said Kovács.

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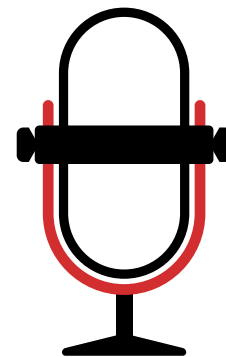
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Time Traveling Mini-brains on a Mission to Conquer Space

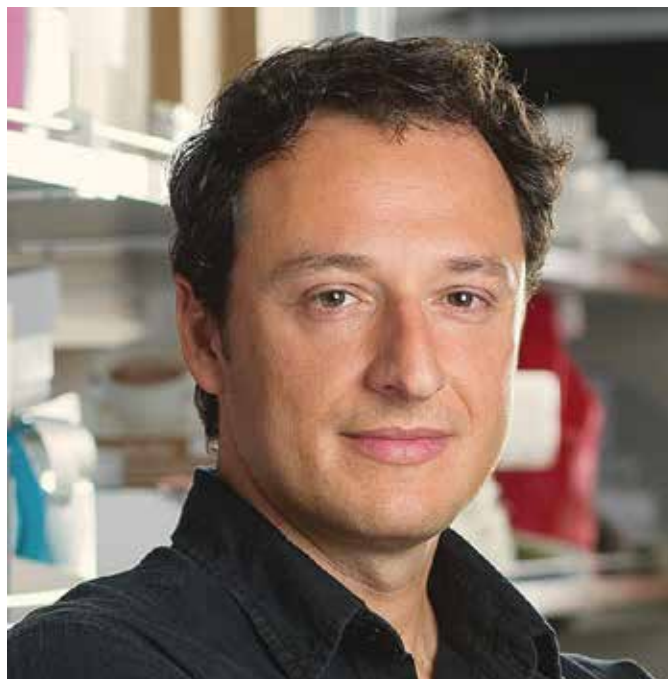
Alysson Muotri launched brain organoids into outer space and found that microgravity enriches our understanding of brain development and disease.

INTERVIEWED BY IRIS KULBATSKI, PhD

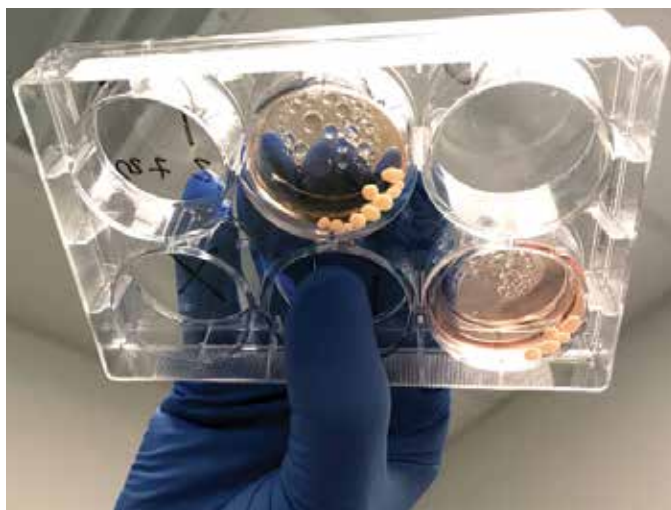
At the University of California, San Diego, Alysson Muotri's research interests break through barriers of space and time. Conjuring scenes from a science fiction film, Muotri studies brain organoids—miniature brains derived from human pluripotent stem cells that exhibit spontaneous neural oscillations, or rudimentary brainwaves. While these organoids mimic human brain development remarkably well, their use as models for studying Alzheimer's disease and dementia is limited by the late onset of these disorders. To overcome the impracticality of waiting decades for brain organoids to sufficiently mature, Muotri sends them on NASA space missions to experience microgravity. When they return to earth, the cells undergo accelerated aging, making them ideal models for brain disorders that manifest later in life. Muotri's space and time traveling mini brains advance scientists' understanding of how aging affects the brain and transform futuristic concepts into reality.

What inspired you to collaborate with NASA?

I began looking for ways to speed up brain organoid maturation and came across literature suggesting that the activity of human telomeres is altered after exposure to microgravity. I thought that space travel may be an intriguing way to age brain organoid cells without having to manipulate them genetically or pharmacologically. I discussed



Alysson Muotri, PhD
Director, Stem Cell Program
Professor of Pediatrics and Cellular & Molecular Medicine
University of California, San Diego

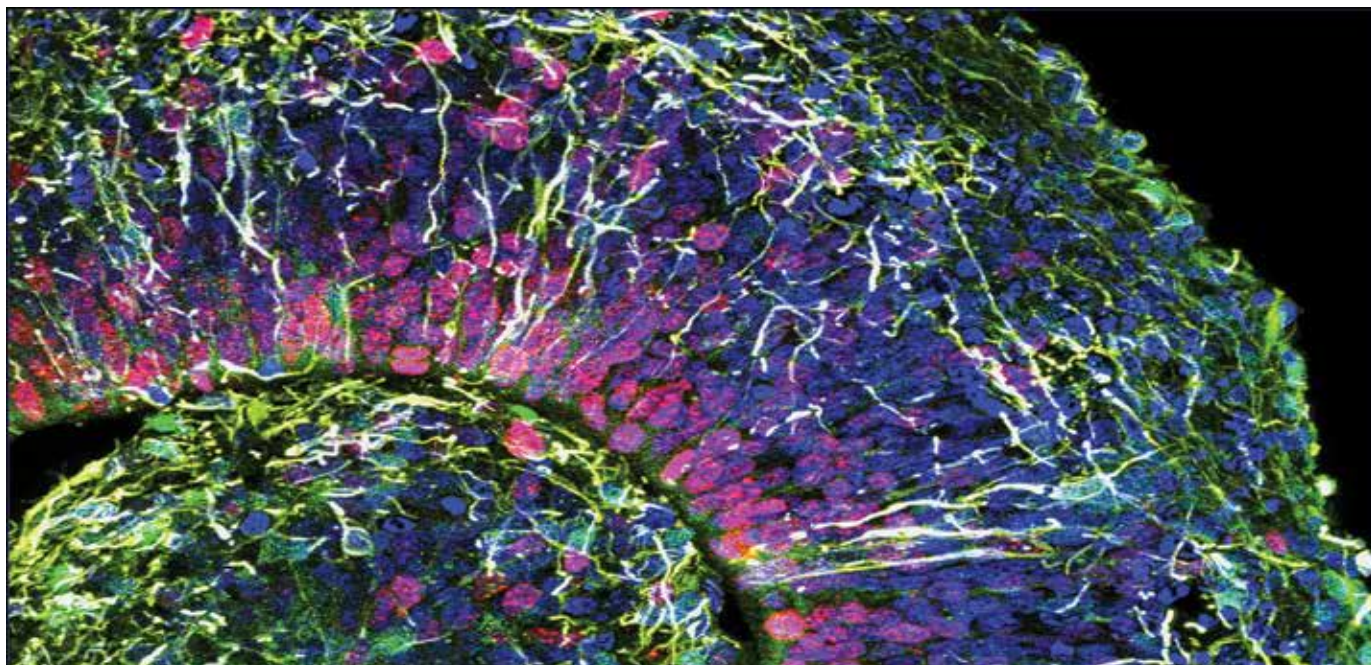


Muotri's team grows brain organoids in a culture dish, then prepares them for lift off in collaboration with NASA.

these ideas with a group of science fiction enthusiasts from the University of California, San Diego. We asked questions like if we colonize Mars in the future, would the brains of human embryos function normally? How would the human brain develop in space? Sending human brain organoids to the space station seemed like an exciting project. It took almost ten years to bring these ideas to fruition because funding agencies were not interested at first. I did not have experience in space research, so the first mission was self-funded. Once we produced results, I received grants for the follow-up missions.

What have you learned from previous space missions?

Our missions usually last one month. When the organoids return, we perform gene expression studies and some histology analyses. In the first mission, we learned that the organoids survive in microgravity.



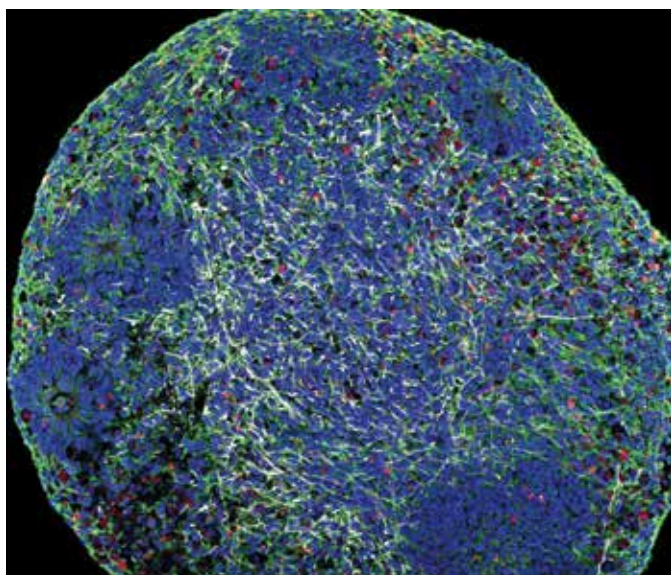
Muotri's team performs high-resolution imaging of immunostained organoids to help them understand how the resident cells age after space missions.

Their shape is different because microgravity affects the migration pattern of the organoid cells during cortical formation. Gene expression after the mission showed signs of cellular senescence, such as increased inflammation, and we found alterations in telomeres as expected. The second mission was designed to validate some of the findings from the first mission. Unfortunately, due to a technical malfunction, we lost contact with the organoids and were unable to feed

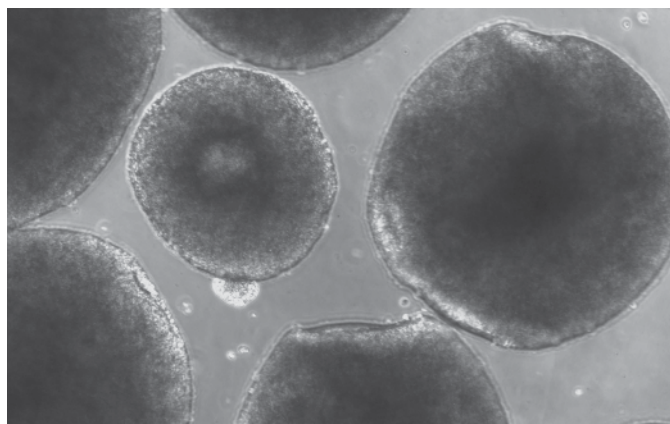
them, so they eventually died. We fixed the system hardware problems to improve reliability for future missions.

What are the challenges of studying organoids in space?

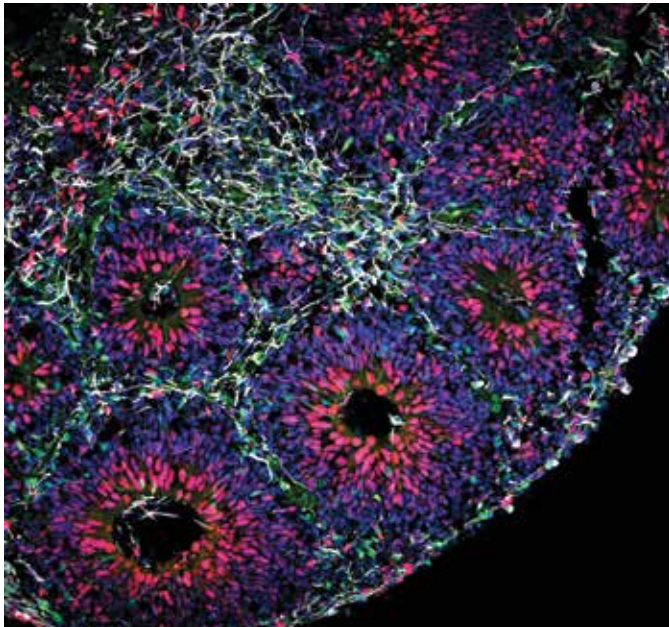
We grow the organoids and optimize their survival within a bioengineered, shoebox-sized system that will sustain them during the space mission. This autonomous system includes a battery, incubator, media replacement mechanism, camera, and microscope. The astronauts do not maintain the organoids. They just plug the system into a power cabinet and at the end of the mission they remove it, place it back in the



Slicing through a brain organoid and performing immunostaining reveals the inner space of mini brains.



Muotri's team uses light microscopy to gain a bird's-eye view of brain organoids and understand their overall size, shape, and density while growing in culture.



Brain organoids are a world all their own. Using them as models for neurodegenerative diseases holds great promise for developing future therapies.

capsule, and we pick it up. We create synchronous controls on earth to replicate the conditions of space flight, for example, shaking the boxes to mimic turbulence on the rocket. We use simulated microgravity as a control, but we cannot continually recreate microgravity for 30 days. It is impossible to replicate space conditions on earth, even with technologies like bioreactors, centrifuges, or other simulators. You can get close

to it, but it's not the same. We also grow several batches of organoids in parallel to accommodate potential last-minute changes in the rocket launch schedule.

What are the broader implications of this work?

This work is a potential game changer for modeling late onset neurodegenerative diseases such as Alzheimer's disease. In terms of cellular aging, we estimate that one month on the space station is equivalent to between 10–30 years on earth. If we can grow organoids in space for six months and then study them for their susceptibility to Alzheimer's disease, we may be able to observe the presence of disease markers such as plaques and tangles and use the organoids to screen drugs. The idea of using the space station as an incubator for aging may be transformative.

What are the ethical considerations for working with brain organoids?

This is all brand new. The ethical considerations are a gray zone now and will likely evolve to include a formal code of ethical conduct as we rapidly increase the complexity of brain organoids. Researchers are now vascularizing the organoids so that they grow larger and exhibit more neural oscillations. They are also attaching retinas to them so they can start receiving visual inputs. As we do these things, we get closer and closer to mimicking the human brain, in which case, the central question becomes, "Have brain organoids reached a level of consciousness where they are self aware and if so, what is their moral status?" ■

This interview has been condensed and edited for clarity.



As researchers advance the complexity of mini brains, they grapple with major ethical questions about consciousness in a culture dish.



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Combating Mosquito-Borne Diseases with CRISPR

As alternatives to insecticides, Omar Akbari uses sophisticated genetic engineering methods to solve the world's mosquito problems.

INTERVIEWED BY NIKI SPAHICH, PhD

Female mosquitoes are some of the deadliest organisms in the world due to their ability to spread infectious diseases through a simple bite. Mosquito-borne diseases such as yellow fever, Zika, Dengue fever, and malaria kill millions of humans every year, and there are limited therapeutics for their prevention and treatment.

While in college, Omar Akbari worked as a public service intern testing the local mosquito populations for human pathogens and eradicating these insects with chemicals. During this experience, he felt dissatisfied with the insecticide-based method of controlling mosquito populations and wanted to find a better way to tackle the problem of mosquito-borne disease spread. With a multidisciplinary team in his laboratory at the University of California, San Diego, he now develops tools through genetic engineering techniques such as CRISPR to solve the world's insect control problems.

How do you create new insect control strategies in the laboratory?

We explore basic developmental biology questions. Once we understand the basic biology and physiology of the insect, then we can manipulate it to develop alternative control technologies. For example, we manipulate a pathway to prevent mosquitoes from transmitting viral diseases. We also develop diagnostics so that people can detect in the field if an insect is infected with a pathogen.

What makes CRISPR a useful tool for your research?

CRISPR is incredibly robust in terms of cutting DNA and targeting nucleic acids. It works really efficiently; we can program it; and it is easy to use. One of the first experiments I did with it was to genetically encode its components in flies and cross those flies together. When I targeted a gene for eye pigmentation or body pigmentation, close to 100 percent of the progeny had some type of mutation in those genes, and I could see the result. That efficiency was a big surprise. We have devised many strategies for using CRISPR to control insect populations in a way that is safe enough to enable public acceptance and regulatory authorization in the near term.

What CRISPR-based strategies for insect control have you developed?

We recently developed a new technology that uses CRISPR to block viral transmission. There are CRISPR ribonucleases that can degrade RNA instead of DNA, and we engineered these to target viruses. We



Omar Akbari, PhD
Professor
Cell and Developmental Biology
University of California, San Diego

encoded the machinery in the mosquito so that they expressed CRISPR ribonucleases and designed guides that target different viruses. When the mosquito gets infected with a virus, the CRISPR machinery cuts the viral RNA sequences, resulting in collateral activity that reduces the mosquito's fitness and ultimately kills the mosquito. Ideally, we want to multiplex the CRISPR machinery to target all mosquito-transmitted viruses that affect humans.

Additionally, we used CRISPRa to overexpress insect developmental genes, which results in complete lethality. We first did this as a proof of concept in flies, and we showed that this works in mosquitoes. CRISPRa uses a version of Cas9 that is inactive (dCas9). It cannot cut DNA, but it progressively binds to targets using guide RNA, which recruits transcriptional machinery to the target promoter region and promotes gene expression. To rescue this overexpression, we mutated the promoters to prevent CRISPRa machinery from binding. We used a creative genetic crossing scheme where we could maintain the engineered mosquito line

in the laboratory by keeping CRISPRa inactive. When we cross these mosquitos with wild type insects that have the targets present, CRISPRa becomes active and causes lethality. This is complete postzygotic isolation, which is the definition of speciation.

Ours is an engineering lab, so we design, build, learn, and iterate until we get something interesting.

—Omar Akbari, University of California, San Diego

What do you see as the most promising technology for insect control?

The sterile insect technique is the most effective way of controlling insects in the wild, but it traditionally uses radiation for sterilization, which reduces insects' fitness due to chromosomal damage. We developed a precision-guided sterile insect technique (pgSIT), which also uses CRISPR. This could be one way of combating mosquitoes that does not rely on insecticides. By using CRISPR, we are not affecting the chromosomes, so the animal is more fit, which results in longer viability in the wild and a higher rate of population suppression.

We make different insect lines homozygous for *cas9* and a guide RNA. We design the guide RNAs to target genes important for female development and male fertility. When we cross lines with *cas9* and these different guide RNAs together, all of the progeny receive the *cas9* gene

and the guide RNAs through Mendelian segregation. CRISPR then targets those genes, and all of the females die and all of the males are sterilized. We want to kill the females because they bite and transmit disease. To control insect populations, one could deploy the progeny into the environment to hatch. When the sterile males try to mate with females, they would produce no progeny, and the population would dwindle.

What excites you about being a scientist?

Our laboratory is very applied, so what excites me is translating the technologies we develop. We are always trying to take on problems that are important in the world. For example, when COVID-19 hit, it was hard to diagnose, which we thought was very rate limiting. We got back into the lab. We wore masks and worked in isolated rooms. And we built a detection system called SENSR that uses CRISPR. We identified a new problem and came up with a solution for it.

Right now, we are working on mosquitoes, but once we can deploy our technologies that solve some problems associated with mosquitos, we will move on to the next big problem. Ours is an engineering lab, so we design, build, learn, and iterate until we get something interesting. My background is molecular biology, and we have chemists, biochemists, geneticists, and engineers in the laboratory. With those fields combined, we can create really interesting products. ■

This interview has been condensed and edited for clarity.

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A Genetic Predisposition to Vitamin D Deficiency Contributes to Severe COVID-19

Ana Teresa Freitas discussed how individual variation in vitamin D synthesis and metabolism influences susceptibility to upper respiratory viruses.

INTERVIEWED BY IRIS KULBATSKI, PhD

Ana Teresa Freitas transformed her personal health challenges into an opportunity when an unclear diagnosis motivated her to embark on a new research trajectory. Freitas realized that the algorithms she used in her electronic and computer engineering work applied to the analysis of regulatory gene networks. She now uses algorithms to explore the human genome and identify genetic data that inform disease prevention in order to help others in similar medical situations.

Freitas and her colleagues took a unique approach to understanding the genetics of immune response variability to SARS-CoV-2.¹ They defined polygenic risk scores, predictors of disease based on individual genetics, for previously identified polymorphisms in genes involved in vitamin D synthesis, metabolism, and downstream pathways. Freitas and her team compared these polygenic risk scores to serum vitamin D levels and COVID-19 disease severity in almost 500 hospitalized COVID-19 patients in Portugal. They identified certain polymorphisms in the GC gene, which encodes vitamin D-binding protein, as risk biomarkers for vitamin D deficiency and found a correlation between these variables and COVID-19 severity.

How did the idea for this work arise?

My team and I developed MyNutriGenes, a genetic test that analyzes genetic predisposition for metabolic syndrome using polygenic risk scores and informs preventive strategies. This test includes an analysis of the genes associated with vitamin D pathways. After more than 9,000 exams, we realized that the Portuguese population had genetic characteristics that predispose them to vitamin D deficiency, which may account for why almost 70% of our population is vitamin D deficient. At the onset of the pandemic, I discussed this topic with my colleague, Conceição Calhau at the New University of Lisbon. We decided to examine the association between polygenic risk scores for polymorphisms in vitamin D-related genes, vitamin D deficiency, and COVID-19 severity based on previous evidence of an association between vitamin D status and COVID-19 mortality rates.² A higher prevalence of one particular variant of the GC gene in Portuguese people versus Europeans likely contributes to the observed prevalence of vitamin D deficiency.

What are possible applications of this work?

This work highlights the important role of serum vitamin D levels and vitamin D-binding protein in SARS-CoV-2 infection and has implications for novel SARS-CoV-2 biomarker discovery. Vitamin D levels and the GC gene variant can be included as part of a composite score for pre-



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dicting COVID-19 severity, along with other candidate biomarkers. It would be interesting to compare the predictive power of a genetic score with that of a more complex multiparameter score comprising both genetic³ and non-genetic markers using a larger dataset for validation.

This research can also be applied to the study of SARS-CoV-2 disease mechanisms. Neutrophils are abundantly recruited in SARS-CoV-2 infection, but this may not be beneficial to the host. Vitamin D-binding protein acts as a neutrophil chemotactic factor, and genetic variation in its encoding gene impacts COVID-19 outcomes. Therefore, this work stresses the need to further explore the role of neu-

trophils in SARS-CoV-2 infection to understand whether modulating these immune cells could improve outcomes.

In general, the immune response depends on the concerted action of several types of cells. In this study, a gene that would not be considered the first choice in the context of immunity, *GC*, showed an association with the severity of SARS-CoV-2 infection. There was already some evidence of its importance in immune response, and this study reinforced this association with SARS-CoV-2 infection. This gene should be explored in future studies to assess the existence of other variants associated with variability in immune response and also to explore the underlying mechanisms by means of functional studies.

An individual's access to information about their own genetic predispositions enables a personalized approach to disease prevention and treatment.

—Ana Teresa Freitas, University of Lisbon

How might this study shape future clinical practice?

Broadly speaking, this study demonstrates the practical application of preventive genetics for viral diseases. An individual's access to infor-

mation about their own genetic predispositions enables a personalized approach to disease prevention and treatment, which in this case may include increased sunlight exposure or a specific supplementation plan to address vitamin D deficiency. Several European countries, including Ireland and Spain, are already taking preventative measures by defining supplementation programs for COVID-19 and other respiratory viruses.^{4,5} ■

This interview has been condensed and edited for clarity.

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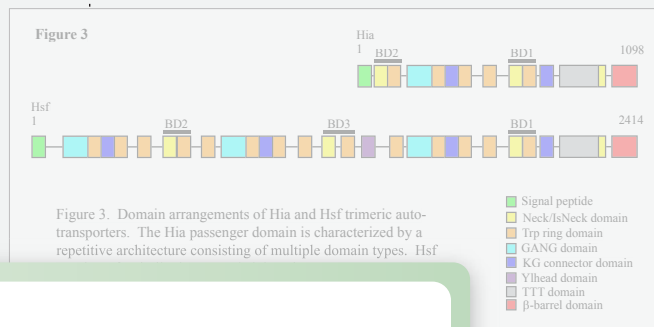
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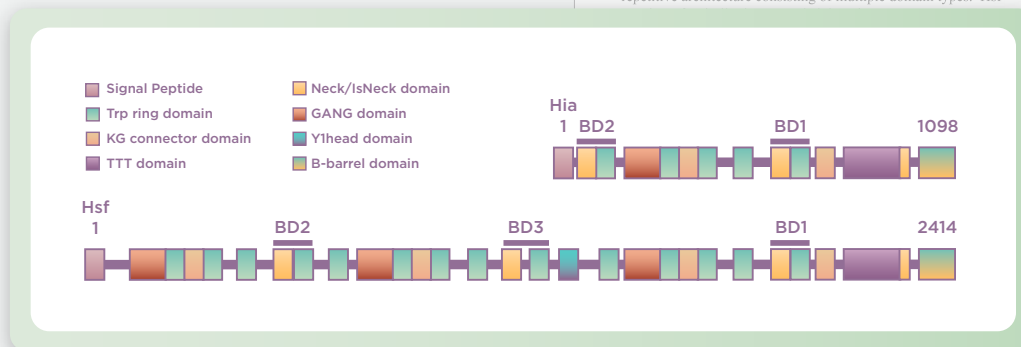
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EDITOR'S CHOICE IN IMMUNOLOGY

Vaccines: Sex Matters

Male patients who recover from mild COVID-19 have baseline immune states primed to mount stronger responses to future challenges than female patients.

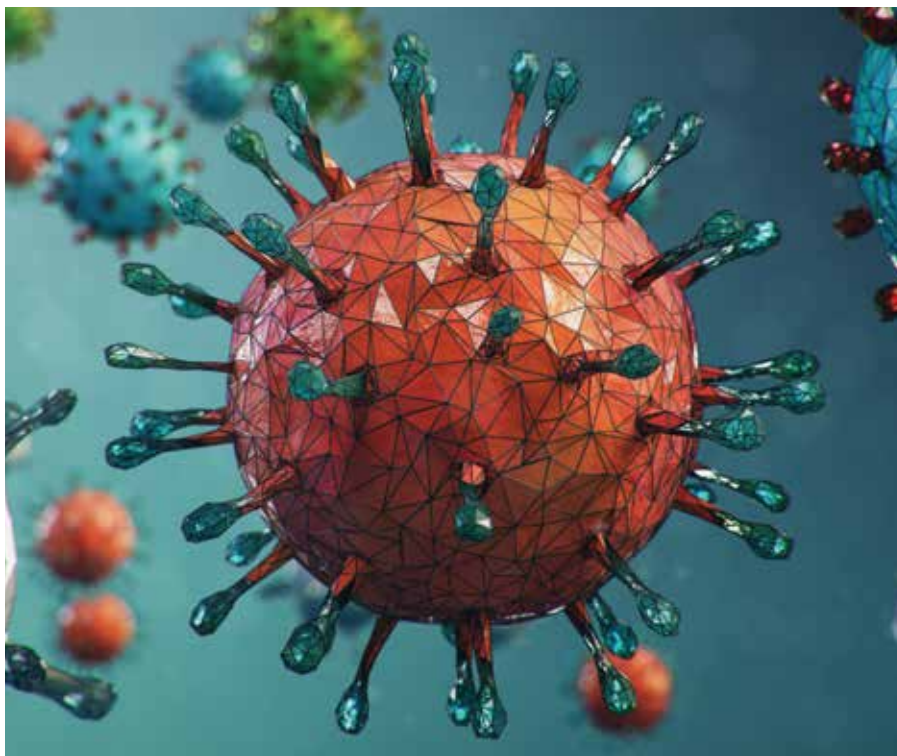
BY NIKI SPAHICH, PhD

When healthy individuals suffer from an acute infection, they expect that their immune systems will ramp up to fight the invading pathogens and then return to their baseline states. The truth is not so simple since people have diverse immune responses that can change throughout their lifetimes, including after surviving infection.¹

John Tsang from Yale University has spent his research career determining why people respond to infection differently. “One of the questions we’ve been trying to understand is, ‘How are [baseline immune] states set?’” said Tsang. “If you look at certain immune cells and their states before they get a perturbation like a vaccine, that can predict their responses independent of things like age, sex, [and] pre-existing immunity.”

While in his previous position at the National Institutes of Health, Tsang and a research team assembled by clinical immunologist Rachel Sparks sought to learn if people’s baseline immune statuses changed in response to mild COVID-19. Their results were recently published in *Nature*.²

The early days of the COVID-19 pandemic presented an unprecedented scenario where many people were infected with a pathogen that their immune systems had never experienced. Tsang and Sparks’s team compared the immune statuses of people who had recovered from mild COVID-19 during the first wave of the pandemic to those never exposed to SARS-CoV-2.



After performing multiomic analyses on cells, proteins, RNA, and DNA in the blood to determine each person’s baseline state, the researchers activated the participants’ immune systems by administering flu vaccines. The team then compared the innate and adaptive immune responses over time of male and female volunteers who had or had not experienced COVID-19.

Female participants tend to mount stronger immune responses than male

participants in general and to the flu shot, but Tsang’s team saw the opposite in the COVID-19 recovered population. “We saw that the males had higher antibody responses and corresponding B cell responses to the flu vaccine than females,” said Tsang.

These findings were in line with what other researchers had noticed about COVID-19: male patients tended to have stronger inflammatory responses to SARS-CoV-2 infec-

We saw that the males had higher antibody responses and corresponding B cell responses to the flu vaccine than females.

—John Tsang, Yale University

tion and greater mortality rates than female patients.³ Tsang hypothesized that the robust reaction in male volunteers may leave lasting effects on multiple immune cell types.

The researchers found that male patients who recovered from COVID-19 had elevated numbers of long-lived virtual memory T cells at baseline. These cells have not been exposed to their target antigen, yet respond vigorously to

cytokine stimuli, similar to true memory T cells. After vaccination, COVID-19 recovered male individuals exhibited monocyte populations that highly expressed the inflammatory cytokine interleukin 15 (IL-15), which induced virtual memory T cells to produce another cytokine, interferon gamma (IFN- γ). These elevated cell numbers and cytokine levels may be what caused greater antibody production against influenza vaccines.

“There’re so many possible biomarkers that have been proposed in the literature, but at the end of the day, we always struggle with a question of association and causation. I think one of the nice things about this paper is that we are getting closer to trying to look at this mechanistically,” said Grace Lam at the University of Alberta, who was not involved in

the study. “That suggests that there is an avenue for intervention.”

Tsang hopes that these findings will help researchers build better vaccines. By adding IL-15 as a vaccine adjuvant, individuals—whether male or female—could mount stronger immune responses with increased antibody production in response to vaccination. ■

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EDITOR'S CHOICE IN NEUROSCIENCE

Mouse Brain Cells Activate When They Witness a Fight

A subset of hypothalamic neurons mirrors aggression in mice, challenging previous views on the location and functional role of these cells.

BY MARIELLA BODEMEIER LOAYZA CAREAGA, PhD

Whether it's a lioness aggressively protecting her cubs or a chimpanzee defending his territory against unfamiliar rivals, life in nature is not always peaceful. The reasons animals clash with each other may vary, but one aspect holds true: Fights are rarely a private affair in the wild. Often, there are spectators.

Nirao Shah and his team at Stanford University wondered how those individuals on the sidelines of a fight perceive aggressive interactions. Their findings, published in *Cell*, describe a subset of neurons in the hypothalamus that fire when a mouse fights or watches other mice fight, indicating the existence of mirror neurons, cells that are active when an animal performs and observes a behavior. Their results also revealed that these cells trigger aggression, pointing to a functional role of these neurons.¹

Shah's team previously identified a cluster of neurons in a brain region called the ventrolateral part of the ventromedial hypothalamus (VMHvl) that triggered aggression in mice. Those findings have been replicated by other researchers over the years, providing strong evidence that the VMHvl is the rage center of the brain. A few years later, though, his team made the surprising observation that stimulating these rage cells in group-housed mice did not induce attacks, suggesting that social context could regulate the activity of these neurons.

To investigate this idea, the researchers recorded the activity of the VMHvl cells in mice under two conditions: when they participated in a fight or when they witnessed other mice fight.



The team introduced a male mouse into another's cage to initiate a fight. The resident mouse attacked the intruder and displayed threatening behaviors, such as tail rattling, to defend its territory. To set up an observer, the researchers placed a

Like many animals, field mice (*Apodemus agrarius*) fight to protect their territories.

mouse in an adjacent compartment separated from the fight arena by a transparent divider. The researchers found

that the same population of VMHvl neurons that fired when mice fought also activated when they observed others fight, indicating that these cells mirror aggressive behavior.

Next, they wondered whether these neurons trigger aggression. To find out, the team used genetically engineered mice that allowed them to stimulate or inhibit the cells' firing. "If we silence these neurons, aggression is basically gone, but mating with females is unaffected, [suggesting that] mirroring neurons are required for aggression, but not for another equally important social behavior. When we activate these neurons, males attack other males more viciously. Instead of mating a female, they attack females, and if you give them a mirror, they will attack their own image," Shah said.

Most evidence for this particular nucleus of the hypothalamus is related to the generation of the behavior, not necessarily anything cognitive. And mirror neurons are a cognitive ability.

—Nancy Padilla-Coreano,
University of Florida

Nancy Padilla-Coreano, a neuroscientist at the University of Florida who was not involved in the study, said that the findings broaden the understanding of the hypothalamus's function. "Most evidence for this particular nucleus of

the hypothalamus is [related to] the generation of the behavior, not necessarily anything cognitive. And mirror neurons are a cognitive ability."

She also suggested investigating if this phenomenon is sexually dimorphic as a next step. "[Females] are aggressive in certain conditions as opposed to males who seem to need less reason to be aggressive. If the mirror neurons are only in the males, that could suggest that mirror neurons are a function of the higher baseline of aggression."

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Cellular Competence: Making Recombinant DNA Accessible

Coaxing bacteria into taking up recombinant DNA was arduous until Douglas Hanahan took action.

BY NATHAN NI, PhD

Recombinant DNA technologies developed in the early 1970s enabled scientists to isolate genes of interest and splice them into existing DNA structures. However, as late as the early 1980s, the molecular cloning process was horribly inefficient because it was difficult to integrate isolated genes with existing genomes. These challenges were unacceptable to Douglas Hanahan, currently director emeritus of the Swiss Institute for Experimental Cancer Research (ISREC) in the Swiss Federal Institute of Technology Lausanne (EPFL). To overcome them, he pioneered a new transformation method that now bears his name.¹

In 1979, Hanahan joined Paul Doty's laboratory at Harvard University to study techniques for cDNA cloning. "The goal was to first make recombinant DNA, convert it into cDNA using reverse transcriptase, then put it into plasmids, transform *E. coli*, and finally scale that up," said Hanahan. However, his early forays met little success.

To complicate matters, Hanahan was caught in the midst of an ethical furor within the scientific community regarding the safety of recombinant DNA technology. "People were developing these techniques and realizing the power that they had to create any new gene," said Rosemary Redfield of the University of British Columbia. "Scientists quickly said, 'are we sure we want to do this?'" In particular, there was a fear that genetic material, once placed inside a host organism, might escape the laboratory and spread uncontrolled. People saw *E. coli* as a particular threat since it naturally populates the human intestinal tract.

"When I joined Harvard, recombinant DNA and cloning experiments had to be done in very specialized physical containment facilities that had only been used for very pathogenic viruses up until that point," Hanahan recalled. Not only did Harvard University lack such a facility, but the city of Cambridge, Massachusetts had placed a moratorium on such research. Hanahan and his colleagues had to travel to Cold Spring Harbor Laboratory—a 225-mile journey—to do their work. "We'd drive down there, gown up, go into this special facility, and we basically got nothing. There were no positive colonies. This was pretty depressing."

Hanahan started playing around with isolated plasmids and adding different things to cultures. He soon recognized that calcium chloride—a divalent cation—rendered *E. coli* more permis-



Doug Hanahan worked at Cold Spring Harbor Laboratory in 1982.

sive to DNA uptake, although the process was still inefficient. Sparked by this discovery, he started experimenting with other metal ions, as well as various combinations of ions, agents, and other factors such as plasmid sizes.²

"They were just empirical iterative combinatorial studies, mostly involving cocktails of divalent or multivalent ions. But rendering *E. coli* competent really enabled molecular cloning to be feasible, and so the method was obviously widely adopted," said Hanahan. He eventually improved DNA uptake efficiency by ten-thousand-fold or more.

Hanahan's method for improving DNA uptake played a large part in making recombinant DNA technology accessible. "Competent *E. coli* have become a mainstay across the life sciences," Hanahan noted. "It spawned a whole research product industry with all of these different companies following my protocols and producing competent *E. coli* to sell to others." ■

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A Not-So-Simple Idea

A moonlit drive inspired the theory behind PCR. What did it take to make that wild idea reality?

BY NIKI SPAHICH, PhD

Today, PCR is ubiquitous in molecular biology laboratories. As one of the first experiments that young scientists master, it is easy to take for granted. But developing this research lab staple required a team of scientists painstakingly repeating trials and troubleshooting parameters to finally amplify a single, small, targeted DNA sequence.

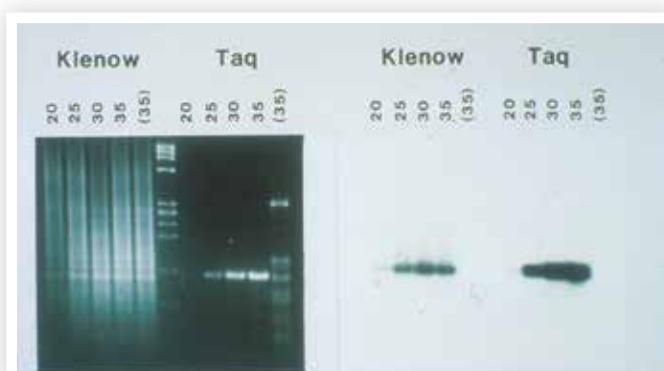
“Once you’ve shown that it works, this idea is kind of simple and almost seems, in retrospect, obvious,” said Henry Erlich, currently a senior scientist at the Children’s Hospital Oakland Research Institute. “But the idea that you could amplify a specific targeted DNA sequence using an oligonucleotide primer and DNA polymerase was not at all obvious.” Erlich led the team of scientists at the Cetus Corporation that developed the PCR method.

In the 1950s, scientists discovered *Escherichia coli* DNA polymerase and immediately put it to use synthesizing DNA molecules for many research purposes. By adding a complementary oligonucleotide primer, scientists could control where DNA synthesis began, but not where it ended. Complex sequences such as those in the human genome contained multiple places where a single primer could hybridize, leading to a complete lack of specificity in the end products. Kary Mullis, a chemist in the department of human genetics at Cetus Corporation, decided to solve the primer specificity problem.

During a night-time drive along the California coast in 1983, an idea that would revolutionize molecular biology struck Mullis. By building upon the simple DNA synthesis and sequencing techniques already in use, Mullis realized that he could achieve targeted DNA synthesis by adding a second primer to hybridize with the end of a desired sequence. As legend has it, further into the drive, Mullis realized that performing multiple cycles of the polymerase reaction with two primers would exponentially amplify the target sequence.¹

Mullis eagerly took his novel idea to the lab and recruited his research assistant, Fred Faloona, to attempt the reaction. After combining the necessary components into a test tube—template DNA, nucleotides, primers, and polymerase—they were disappointed to observe a large smear after performing gel electrophoresis on the completed reaction. Mullis was convinced that his method worked, but other scientists were highly skeptical.

Tom White, then head of research at Cetus Corporation, saw potential in Mullis’ idea and asked Erlich and the human genetics team he led, including Randy Saiki, Steve Scharf,



This original gel image shows amplification of the 110 bp β -globin fragment using either Klenow or Taq DNA polymerases. The band can be seen in the gel electrophoresis results (left) and Southern blot (right).

and Norm Arnheim, to work in parallel with Mullis. The team attempted to amplify the same fragment that Mullis targeted—a 110 base pair segment from the human β -globin gene. At first, they could not determine if they achieved specific target amplification; their gel electrophoresis results were also large smears. They needed to determine if their product was part of this smear.

Over time, the team experienced a series of minor celebrations as they inched closer to the PCR method we are familiar with today. A crucial moment occurred toward the end of 1984 when Erlich and Saiki performed a Southern blot on their smeared DNA sample using a radioactive probe complementary to the β -globin fragment. Erlich and his team were extremely excited by the result; the blot showed a distinct radioactive band at 110 bp. “We thought, ‘Oh my god, this thing could actually work,’” Erlich recalled.

The team next tackled how to improve the reaction’s efficiency. “Only about 1 percent of what was synthesized in that tube was β -globin,” said Erlich.

From the beginning, the Klenow polymerase the scientists were using was problematic. It degraded at 94°C, the temperature required to denature the DNA target; this meant that the scientists had to open the PCR reaction tube at the start of every cycle to add fresh enzyme. Additionally, Klenow worked best at 37°C, a temperature that was too low for the primers to bind precisely to their complementary sequences. “The PCR primers hybridized to various places in the genome and



Henry Erlich led the human genetics team that developed the PCR method.

that was the reason that the system wasn't operating as diagrammed on a blackboard. We were getting nonspecific priming, and therefore we were synthesizing a lot of DNA that was not the target," explained Erlich.

The idea that you could amplify a specific targeted DNA sequence using an oligonucleotide primer and DNA polymerase was not at all obvious.

—Henry Erlich, Children's Hospital Oakland Research Institute

Still dedicated to making the technique work, Cetus Corporation scientists David Gelfand and Susanne Stoffel purified a thermostable DNA polymerase from *Thermus aquaticus*—the now-famous Taq polymerase—which survives denaturing temperatures and copies DNA at 70°C.² "We no longer had to add enzyme at every step," said Erlich. "That was actually a major breakthrough in the development of PCR technology—that you could just put everything in. You didn't have to keep adding enzyme and opening the tube. You could just put everything in the tube, close it, and cycle the temperature, and PCR would occur."

Additionally, raising the annealing and elongation temperatures increased primer binding specificity. "With this

thermostable DNA polymerase, if you just ran a gel with the whole reaction, you'd see a discrete band at the expected position in the gel for a 110 base pair fragment," Erlich explained. "If you used a [detection] probe, that was clearly β -globin."

At this stage, researchers changed the reaction temperature by manually moving tubes between water baths set at different temperatures, a procedure that was time consuming and not sustainable long term. Cetus Corporation engineers built a crude thermocycler with protruding tubes and piping to change the temperature in the PCR vessel. "We called it Mr. Cycle, after the then popular Mr. Coffee," Erlich remembered. After the addition of Taq to the protocol, Mr. Cycle needed an upgrade to progress more quickly through the reaction temperatures. Thanks to a joint venture with the Perkin Elmer Corporation in 1985, the first modern PCR thermocyclers were born.

With an automated PCR reaction that faithfully amplified target DNA now consistently working, the Cetus Corporation scientists quickly applied the technology to many diverse projects. Almost immediately, they added restriction sites to the primers to facilitate cloning reactions. Russ Higuchi developed quantitative PCR and John Sninsky quickly adapted the technique to detect HIV viral loads, which was instrumental during the AIDS epidemic. PCR was also a game changer for Erlich's own work characterizing individuals' polymorphic HLA genes. This led to PCR's first appearance in the courtroom in a criminal case where Erlich reported detecting HLA in degraded DNA samples. A year later, he used PCR for the first time in a postconviction exoneration.

"The idea that some basic research that I had been doing could be used in the area of criminal justice and have a practical and significant effect was very, very exciting," said Erlich. "To be honest, we all thought this was going to be a big deal. But I think it's turned out over the last 35 years to be an even bigger deal than we imagined." ■

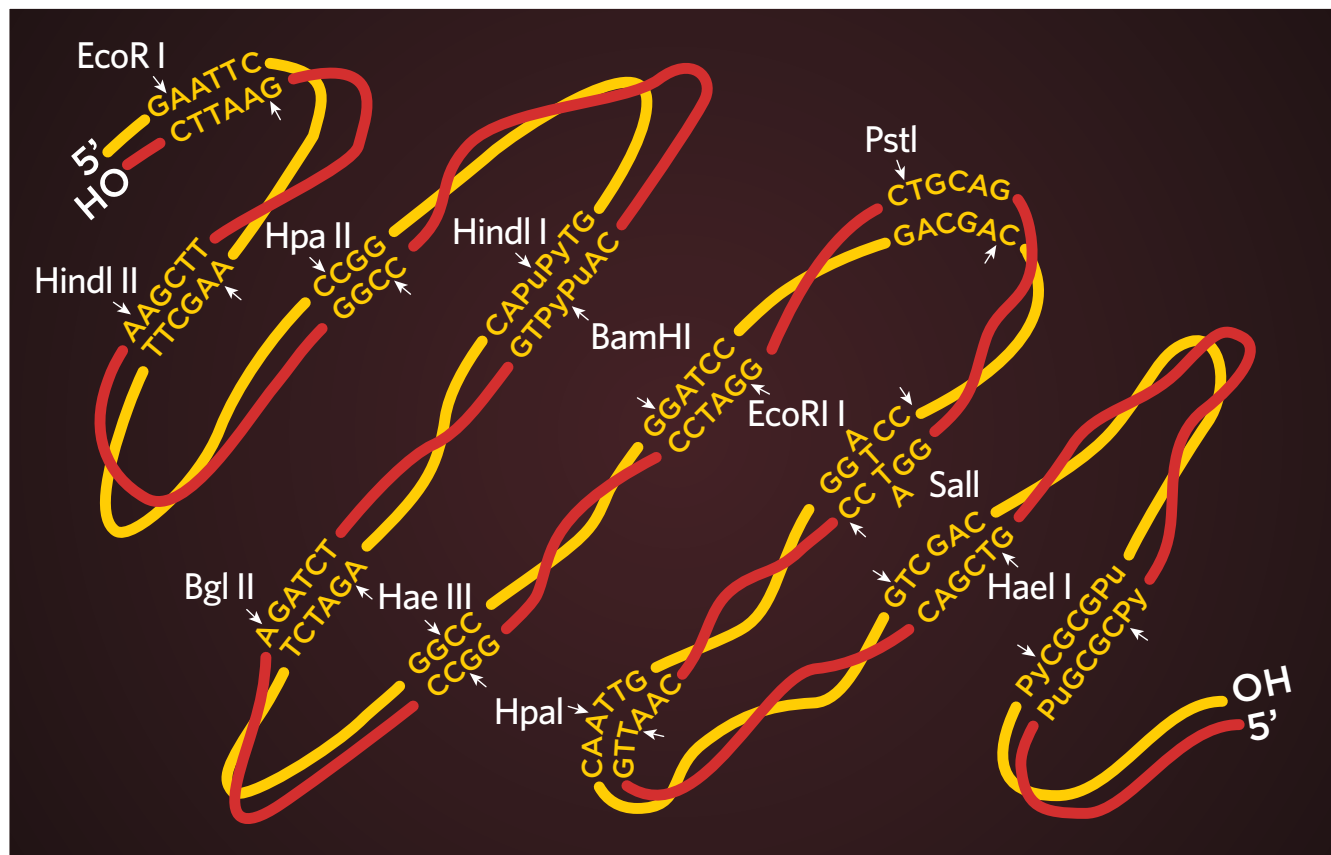
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How Restriction Enzymes Changed Biology

Endonuclease R changed Richard Roberts' career trajectory and created an industry.

BY NATHAN NI, PhD



This diagram shows cleavage site sequences for commonly used restriction enzymes.

In the early 1970s, researchers reported that restriction enzymes cleave DNA at specific sites, producing individual fragments that are distinguishable using gel electrophoresis.^{1,2} This timely discovery opened a new window for understanding and manipulating DNA, but for 1993 Nobel laureate and chief scientific officer at New England Biolabs, Sir Richard Roberts, it began a lifelong passion for restriction enzymes and their potential.³

Scientists first observed the biological phenomenon of restriction and modification in the 1950s, but they did not identify and isolate restriction enzymes until the late 1960s.³ In 1970, Hamilton Smith and Kent Wilcox from Johns Hopkins University discovered “endonuclease R” (later named HindII), a restriction enzyme isolated from *Hemophilus influenza*.¹

A year later, Kathleen Danna and Daniel Nathans, also from Johns Hopkins University, found that endonuclease R produced specific fragments of simian virus 40 and that gel electrophoresis could distinguish these fragments from each other.

Richards first encountered endonuclease R during his postdoctoral fellowship at Harvard University in 1972 when he attended a talk by Nathans. “In the early days, you couldn’t do much with DNA because it was a very large molecule,” Roberts recalled. “As soon as the Type II restriction enzymes were discovered—and HindII in particular—it was obvious that you could take a fairly large DNA and cut it into smaller pieces. And that offered you the opportunity to look and see what was going on.”



Sir Richard Roberts won the 1993 Nobel Prize “for their discoveries of split genes.”

Later that same year, Roberts joined James Watson’s team at the Cold Spring Harbor Laboratory and immediately began isolating endonuclease R and other restriction enzymes. “Every time we opened up a new bug, there was a restriction enzyme with a new specificity,” Roberts said. “People used to bring test tubes with DNA samples to meetings, and they’d step out and ask if we had any enzymes that would cut their DNA.”

While Roberts pursued new restriction enzymes, others used them to sequence, map, clone, and manipulate genes. At the start of his tenure with Cold Spring Harbor Laboratory, Roberts originally intended to focus on using restriction enzymes to sequence DNA, echoing the RNA sequencing techniques developed by Frederick Sanger half a decade prior. “One of the reasons that [sequencing] methods had been developed for RNA research was because there were small molecules to practice on. Restriction enzymes gave the opportunity to do likewise for DNA,” said Roberts. However, he and his team soon found restriction enzymes themselves to be so interesting that they abandoned their foray into sequencing. Eventually, Roberts’ team sent restriction enzymes to Sanger, helping him develop the DNA sequencing technique that bears his name today.

Roberts’ team characterized or discovered three-quarters of the world’s first restriction enzymes.⁴ In the absence of

commercial sources, scientists purified restriction enzymes in their own labs—or came knocking at Roberts’ door. As world-wide demand increased, Roberts encouraged Cold Spring Harbor Laboratory to commercialize restriction enzyme production for the benefit of the scientific community, but he encountered opposition from Watson, who did not see potential for profit in it. Fortunately, Roberts crossed paths with Donald Comb, a previous faculty member at Harvard University Medical School who had just started a small company called New England Biolabs (NEB),⁵ in 1974. At the time, Comb intended to sell HindII through Miles Laboratories. This naturally piqued Roberts’ interest, and he contacted Comb and convinced him to sell restriction enzymes directly using NEB instead. A relationship formed, and Roberts quickly became the chief consultant for the fledgling company that he now calls home.

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—Sir Richard Roberts, New England Biolabs

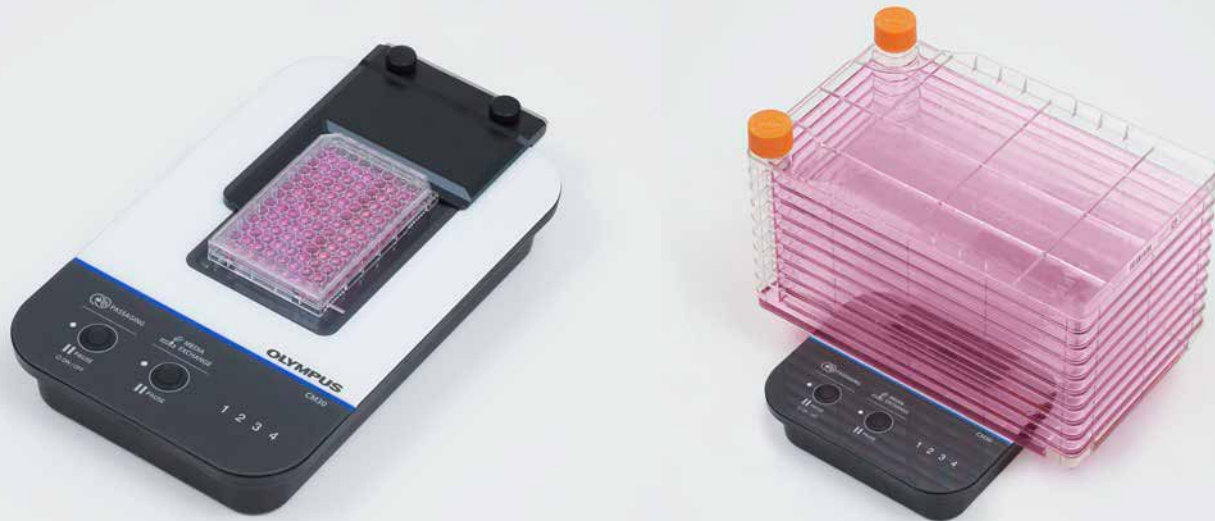
“At the start, restriction enzymes were very important in isolating DNA fragments. These days, they’re more often used to diagnose whether you made the recombinant DNA properly or not,” Roberts said. “There was a time when PCR came along that people thought that the market for restriction enzymes was going to disappear, but what they failed to realize was that PCR just meant that there was going to be a lot more DNA around. All of a sudden, as people sought to check their sequences for accuracy, the market changed, but demand went up.” ■

References

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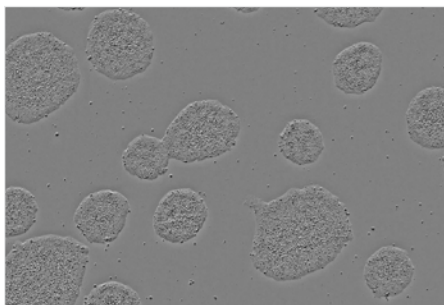
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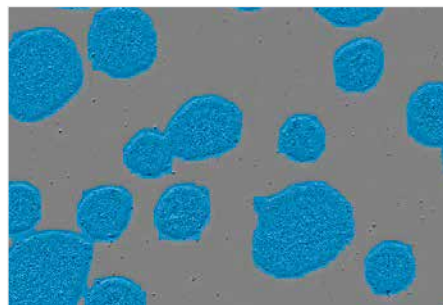
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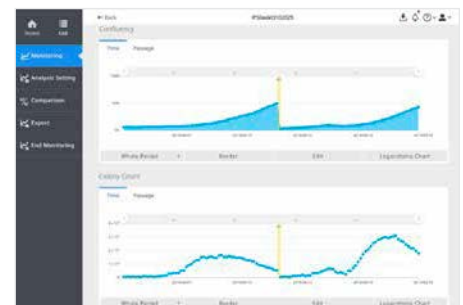
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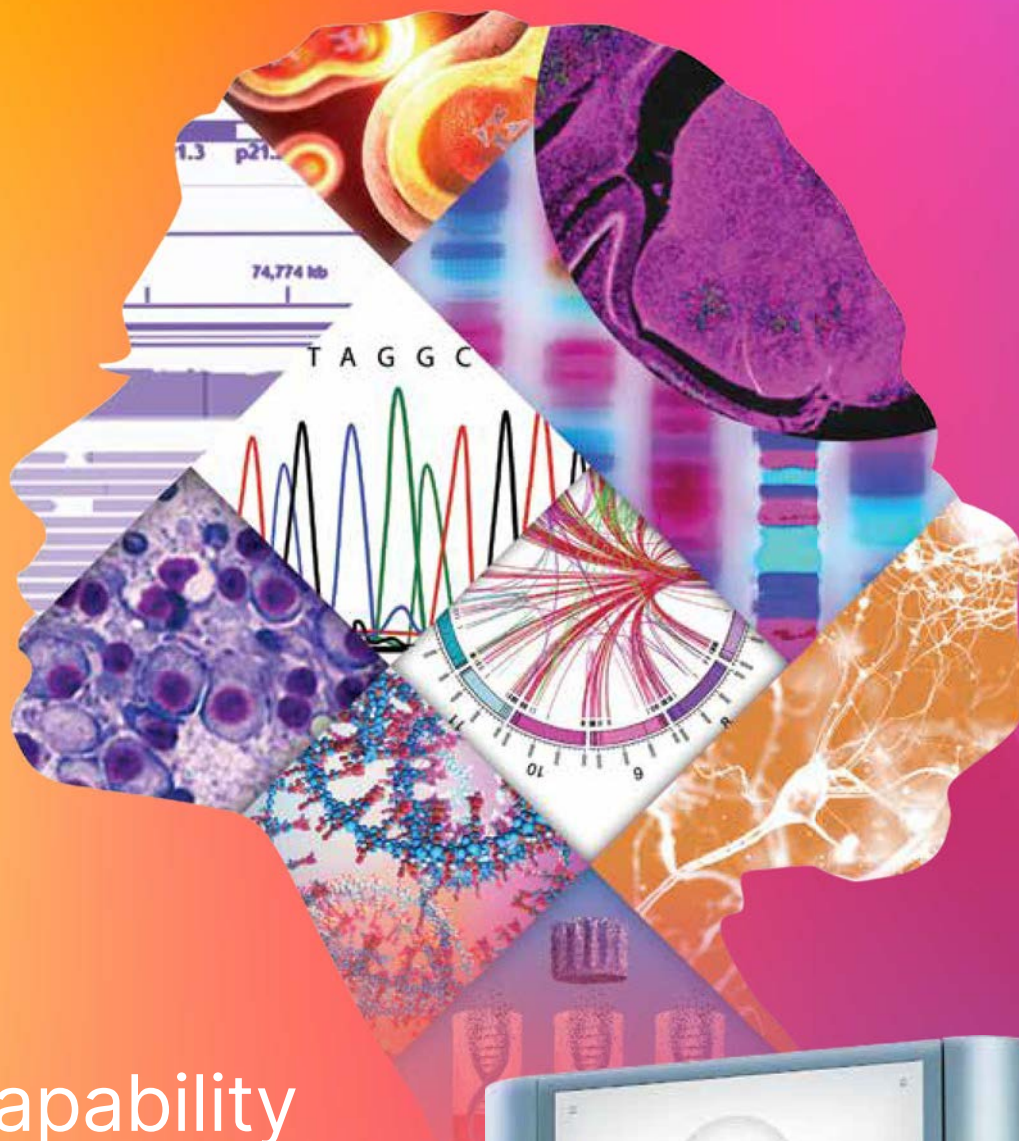
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