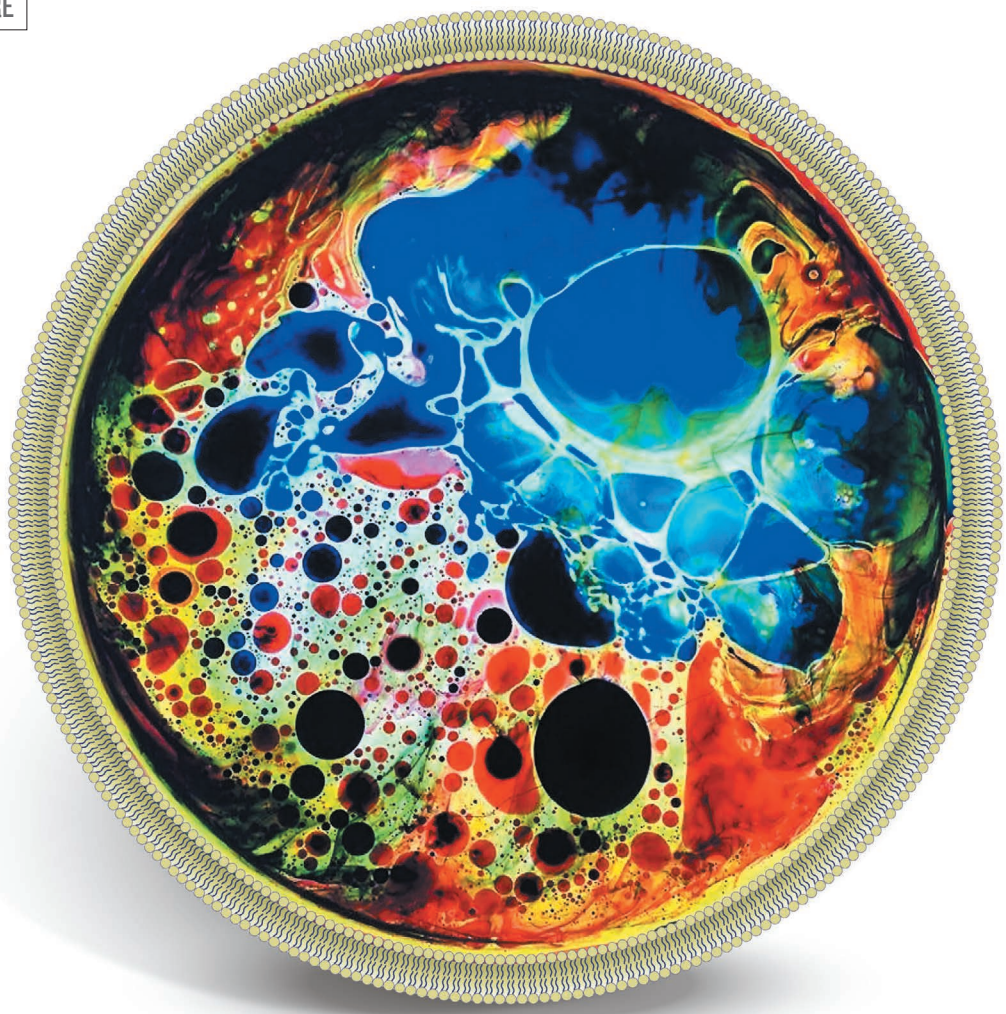


Cell biology's new phase

Like oil in water, the contents of cells can separate into droplets. Finding out why is one of biology's hottest questions.

BY ELIE DOLGIN

When David Courson and Lindsay Moore arrived for a summer research placement in Woods Hole, Massachusetts, they expected to try some new techniques and play with high-end microscopes. As graduate students, they never imagined that they would help to solve a biological problem that had baffled researchers for more than 25 years.

Their instructors at the Marine Biological Laboratory asked them to decipher how pellets of RNA and protein called P granules form in worm embryos — a tall order given how long the structures had flummoxed biologists. Yet as soon as Courson and Moore started making movies of the process, they and their instructors could see something unusual happening under the microscope: the P granules were colliding and coalescing like blobs in a lava lamp.

Solid structures don't do that; only liquids can. The P granules, they realized, were not hard kernels, as most researchers thought. Rather, they behaved like oil droplets in a bottle of vigorously shaken vinaigrette, first dispersing, then quickly fusing and blending into larger liquid blobs.

This process is a bread-and-butter concept in engineering, chemistry and physics, called liquid–liquid phase separation. It occurs whenever there's a force pushing two liquids apart, as when oil floats on top of water. Phase separation is common in nature and crucial in many industrial processes. Still, it wasn't an idea that Courson, a cell biologist now at Old Dominion University in Norfolk, Virginia, had come across. When he saw the P granules fuse like liquids, "it was a really neat moment", he says, "but I didn't understand the scope or the scale of it".

There was no more time to examine the process on the short summer course. But when the instructors, cell biologist Tony Hyman and his postdoc, biophysicist Cliff Brangwynne, returned to their lab at the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden, Germany, they ran some more experiments: they stuck worm gonads filled with P granules between two thin plates of glass and slid the plates past each other. Under the shear stress of the sliding plates, solids would smear out, but the granules merged, dripped and beaded up like rain drops on an umbrella.

That's when the magnitude of the discovery dawned on them. Phase separation might provide a way of concentrating certain molecules and excluding others to create order in the crowded chaos of the cell — an organizational feat that Hyman says biologists hadn't considered in any formal, quantitative way.

OIL ART: STEVE PAVLOVSKY/LIQUID LIGHT LAB

"It was just one of those questions people hadn't thought to ask," he says. Hyman and Brangwynne published their results[1] in 2009.

In the ensuing decade, scientists around the world have jumped on the idea that phase separation can explain how cells partition the molecules swarming inside them. These biological droplets could provide crucibles to speed up reactions, or quarantine unwanted or unneeded factors. "It's one of these in-hindsight, intuitive ideas. The second you hear it, it just makes a lot of sense," says Shana Elbaum-Garfinkle, a biophysicist at the City University of New York Advanced Science Research Center in New York City.

Not only is phase separation intuitive, but it seems to be everywhere. Droplets of proteins and RNAs are turning up in bacteria, fungi, plants and animals. Phase separation at the wrong place or time could create clogs or aggregate of molecules linked to neurodegenerative diseases, and poorly formed droplets could contribute to cancers and might help explain the ageing process (see 'Separate ways'). "It's a new paradigm that's really transforming our understanding of cell biology as a whole," says Elbaum-Garfinkle.

Yet some researchers think it's too early to say whether phase separation plays a major part in organizing the cell and causing disease. They suggest that it could simply be a side effect of chemical interactions, with little impact on cellular mechanics. Just because researchers can think of how a cell might use phase separation, it doesn't mean it's definitely happening, says Tim Mitchison, a cell biologist at Harvard Medical School in Boston, Massachusetts. "Those are just ideas right now. That's not really proof."

Researchers want that proof. "This is the multimillion-dollar question at this point," says Rohit Pappu, a computational biophysicist at Washington University in St. Louis, Missouri. "Is this some sort of by-product of sticky molecules being produced by the cell? Or did nature figure out how to use this advantageously?"

DROP BY DROP

As far back as 1899, US cell biologist Edmund Beecher Wilson anticipated that the main bulk of a cell, the cytoplasm, might include "a mixture of liquids" with "suspended drops … of different chemical nature"[2]. By the 1990s, researchers were beginning to speculate that phase separation might underlie disease or offer a general organizational principle in the cell.

These theories remained on the fringe, however. "It was mostly hypothetical," says Harry Walter, a retired chemical biologist who spent his career at the Veterans Affairs Medical Center in Long Beach, California. "It seemed logical that it should happen, but there was no scientific proof."

Some biologists had observed phase separation in specific, artificial circumstances — for example, while preparing proteins for X-ray crystallography studies. But few had paid much attention to the phenomenon, or considered how it might relate to the formation of cellular compartments without borders.

Brangwynne and Hyman's 2009 report on worm P granules therefore came as a surprise — and initial reactions varied. Among worm biologists, "it ranged from those who thought it was total BS to those who thought that his group finally described the true nature of P granules", says Dustin Updike, who studies granule function at the MDI Biological Laboratory in Bar Harbor, Maine. And outside that research community, most scientists basically ignored it. Fairly quickly, however, came solid evidence that phase separation in the cell was real.

> *"The second you hear it, it just makes a lot of sense."*

In 2011, Hyman, Mitchison and Brangwynne — who set up his own lab at Princeton University in New Jersey that year — showed[3] that the nucleolus, a dense cluster of genetic material and proteins in the cell nucleus, also exhibited droplet-like behaviours. A year later, independent groups led by structural biologist Michael Rosen and biochemist Steven McKnight, both at the University of Texas Southwestern Medical Center in Dallas, studied collections of proteins and RNA molecules in test tubes and found[4,5] that the molecules were weakly attracted to each other, forming droplets and jelly-like blobs.

These 2012 studies, unlike Brangwynne and Hyman's earlier work, showed that phase separation could be reproduced in test tubes with fairly simple biochemical recipes. That made it a lot easier to study in the lab, Rosen says — and from there, "the field has exploded".

The boom began in early 2015, when a team led by Julie Forman-Kay, a structural biologist at the Hospital for Sick Children in Toronto, Canada, showed[6] that a protein important for sperm function formed droplets in human cells. Before the year was up, more than half a dozen groups had published papers showing phase separation with their pet proteins. "We called it the flurry," says Elbaum-Garfinkle, who was a postdoc in Brangwynne's lab at the time, and lead author on one of the papers[7].

Several of the proteins probed in the flurry were implicated in disease development. Researchers spotted it in motor neuron disease, or amyotrophic lateral sclerosis (ALS), a neurodegenerative condition characterized by abnormal clumps of protein in the nerve cells that control movement. Studies showed[8,9] that the clumping process began when these proteins joined with other molecules, split from the surrounding cytoplasm and formed droplets. These blobs grew increasingly gummy, ultimately turning rock-hard. "It's like taking room-temperature honey and putting it in the fridge," says Paul Taylor, a molecular neurogeneticist at St. Jude Children's Research Hospital in Memphis, Tennessee, who has documented phase separation in four proteins associated with the disease.

Those were some of the first concrete pieces of evidence that aberrant phase separation that turns liquids into solids might drive disease, says Jim Shorter, a protein biochemist at the University of Pennsylvania in Philadelphia. The process might be needed to partition cells, but when cells overdo it, he says, "they run the risk of forming structures that are perhaps more stable and solid and more difficult to reverse — and that's where you get into trouble".

SEPARATION ANXIETY

Several other diseases could be rooted in faulty phases. Just last month, Susanne Wegmann, a molecular biophysicist at Massachusetts General Hospital (MGH) in Charlestown, and her colleagues described[10] phase separation in the tau protein, which aggregates into tangles in the brains of people with Alzheimer's disease. Phase separation "might be an initial trigger for aggregation", says Wegmann. This finding, she adds, "starts connecting the dots between these different neurodegenerative diseases".

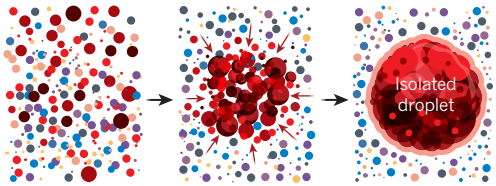
Errors in the phase-separation process could also prompt some cancers. Last year, a team led by MGH molecular pathologist Miguel Rivera identified[11] a protein implicated in Ewing's sarcoma that provokes activity in cancer-causing genes when it gathers near pieces of the genome linked to tumour formation. Aberrant phase separation allows the protein to build up in these areas. And last month, at the annual meeting of the Biophysical Society in San Francisco, California, structural biologist Tanja Mittag from St. Jude outlined how a protein that usually sequesters and destroys cancer-causing molecules inside droplets can instead provoke cancer when mutated, because the droplets no longer form.

These and other reported links to cancer and neurodegeneration prompted Hyman and Simon Alberti, a biochemist at MPI-CBG, to propose[12] that practically any ageing-associated disease could start when cells begin to lose control over phase separation. The body is in a constant struggle to keep its cellular house in order, "and at some point", Alberti says, "the system just breaks down".

But as well as damaging cells, phase separation can help them to adapt. Hyman and Alberti showed[13] this year that when yeast cells are in stressful conditions of low pH, an evolved response triggers one of their essential proteins to form droplets to protect it. The gel disperses only when the pH rises and normal cellular functions can return. This finding dovetails with earlier work from Allan Drummond, a molecular and evolutionary biologist

Separate ways

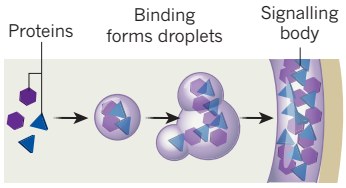
A cell's contents are thought to segregate through a process called phase separation to perform a wide variety of tasks. But flawed phase separation can also cause disease.

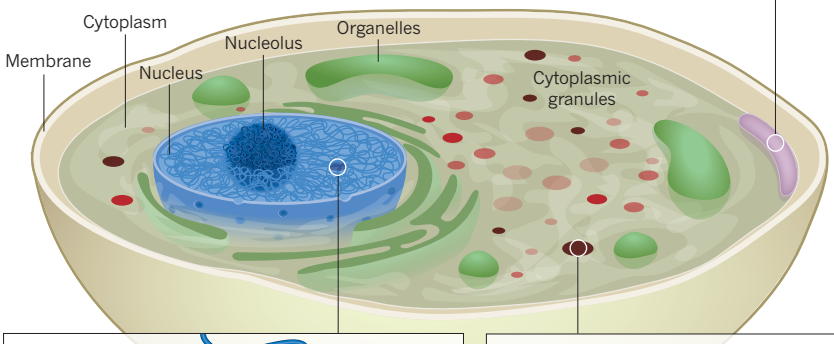


Physical forces between protein or RNA molecules can pull them apart or attract them to each other. Once the molecules reach a certain concentration, they can phase-separate, clustering similar components together to speed up reactions, or sequestering unwanted molecules.

Signalling at the membrane

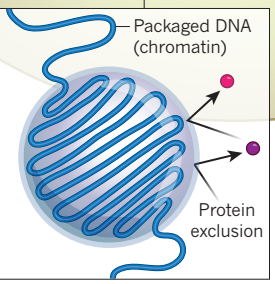
In neurons, proteins necessary for sending signals to neighbouring cells cluster at junctions and phase-separate to ensure smooth communication.





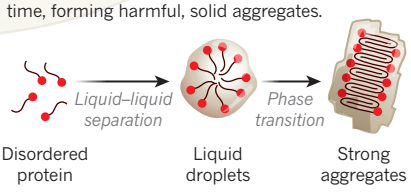
DNA packaging

In the cell nucleus, phase separation helps to compact unused DNA and quell its activity. Some proteins — possibly those involved in transcription — are excluded.



Drops become clogs

In amyotrophic lateral sclerosis, proteins that separate into liquid droplets can congeal over time, forming harmful, solid aggregates.



NIK SPENCER/*NATURE*

at the University of Chicago in Illinois, who reported[14] on a different yeast protein that forms gels as a survival strategy at high temperatures.

Phase separation might thus be a general mechanism by which cells both sense stress and respond to it, says Drummond. "It's like having the alarm also be the thing that turns on the fire hose," he says.

In human cells, forming droplets could be more of an organizational strategy. Last year, biochemist Geeta Narlikar and her colleagues at the University of California, San Francisco, reported[15] that phase separation helps to mothball parts of the human genome that are perpetually inactive and serve mainly a structural function. A team led by structural biologist Mingjie Zhang at the Hong Kong University of Science and Technology found[16] that a piece of cellular machinery that helps brain cells receive signals is built using phase separation.

SHINING A LIGHT

Such studies are beginning to hint at some of the functions of liquid droplets in the cell, but they fail to explain why some components show phase separation, whereas others don't. That frustrates researchers such as Hyman. "We have to define the molecular grammar driving phase separation," he says. And to do that, researchers needed a way of probing, controlling and bending the process to their own will in living cells. As Brangwynne puts it: "We needed tools."

In a dark, windowless third-floor room in a 1970s concrete building at Princeton, Lian Zhu sits hunched by a microscope. A human cell speckled with red blobs lights up her computer screen, each dot denoting a throng of proteins that have phase-separated to form a nucleolus.

Zhu, a PhD student in Brangwynne's lab, fires a blue laser at a spot in the cell, and within seconds new blobs emerge from the black ether. These are fluorescently tagged proteins from the nucleolus fused with a plant protein that, when illuminated with blue light, begins to cling to others of its type. Above a certain threshold, that triggers phase separation[17].

This is what happens in Zhu's cells. The red dots are droplets that appear and dance around the screen before starting to coalesce with others. "It's like a magic trick," Zhu says. By varying the dose of light, Brangwynne and his team can stiffen or loosen various liquid compartments inside living cells, triggering droplets to appear or disappear. Using the tool, Zhu has been working to map the conditions under which nucleolus droplets form, showing how phase separation can occur in one part of the nucleus but fail to materialize in another.

Brangwynne hopes that the tool, dubbed optoDroplet, will bring new rigour to the study of phase separation. "We can now actually approach the level of detail that is standard for non-living materials, where you understand quantitatively what's actually happening," he says. That could be a huge boost for basic biological research, and could help researchers develop drugs by showing how much manipulation is needed to make or break droplets in cells.

Already, some companies are forming to pursue the idea of targeting phase separation to mitigate disease. Earlier this year, for example, a start-up founded by Ron Vale, a cell biologist at the University of California, San Francisco, received seed funding to search for drugs that break up RNA droplets associated with neurodegenerative conditions such as motor neuron disease and Huntington's disease. Taylor is in discussions with investors about starting a company that will identify drug targets using an as-yet unpublished tool — Optogranule — that can recreate the pathology associated with phase separation in cells. The technique allows researchers to watch the neurodegenerative process happening in a dish in a matter of hours.

Others are taking a less guided approach to drug discovery. At MPI-CPG, for example, Hyman and Alberti have blindly screened a small library of approved drug compounds, looking for chemicals that put protein aggregates into a more fluid state. They have identified around 50 candidates. Now they are working out exactly how those drugs affect cellular function.

True progress in the field will require researchers to work out the rules governing how their drops and blobs form — and how to control them, says Brangwynne. "We need to take this to the next level." ■

Elie Dolgin *is a science journalist in Somerville, Massachusetts.*

1. Brangwynne, C. P. *et al. Science* **324,** 1729–1732 (2009).
2. Wilson, E. B. *Science* **10,** 33–45 (1899).
3. Brangwynne, C. P., Mitchison, T. J. & Hyman, A. A. *Proc. Natl Acad. Sci. USA* **108,** 4334–4339 (2011).
4. Li, P. *et al. Nature* **483,** 336–340 (2012).
5. Kato, M. *et al. Cell* **149,** 753–767 (2012).
6. Nott, T. J. *et al. Mol. Cell.* **57,** 936–947 (2015).
7. Elbaum-Garfinkle, S. *et al. Proc. Natl Acad. Sci. USA* **112,** 7189–7194 (2015).
8. Patel, A. *et al. Cell* **162,** 1066–1077 (2015).
9. Molliex, A. *et al. Cell* **163,** 123–133 (2015).
10. Wegmann, S. *et al. EMBO J.* e98049 (2018).
11. Boulay, G. *et al. Cell* **171,** 163–178.e19 (2017).
12. Alberti, S. & Hyman, A. A. *Bioessays* **38,** 959–968 (2016).
13. Franzmann, T. M. *et al. Science* **359,** eaao5654 (2018).
14. Riback, J. A. *et al. Cell* **168,** 1028–1040.e19 (2017).
15. Larson, A. G. *et al. Nature* **547,** 236–240 (2017).
16. Zeng, M. *et al. Cell* **166,** 1163–1175.e12 (2016).
17. Shin, Y. *et al. Cell* **168,** 159–171.e14 (2017).