

## GROUNDBREAKING, NEW VIEW OF CELL FUNCTION EMERGES FROM MBL COURSE RESEARCH

When [Tony Hyman](#) arrived to teach in the Marine Biological Laboratory (MBL) Physiology course in 2008, he didn't imagine that a research project he brought for the students would inspire a major, galvanizing discovery, one that is illuminating the causes and potential cures for a host of serious human diseases.

Accompanying Hyman that summer was postdoctoral fellow [Clifford Brangwynne](#), to be a teaching assistant in the course, and several strains of the worm *C. elegans*, a workhorse species in biological research. The scientists had already fluorescently tagged various proteins in the worms, and the students would observe the proteins and draw conclusions about what they were doing inside the cell.

In one of the strains, they had tagged somewhat mysterious entities called P granules. In *C. elegans* and in other species, when the egg cell is fertilized with sperm, the cell immediately polarizes with clear anterior and posterior ends. The P granules congregate in the posterior (P) end of this new embryo, where eventually sperm and egg cells will form. In 2008, it was unknown how the P granules get to the posterior end of the embryonic cell and what role they might play in creating new reproductive cells.

Brangwynne was sitting with [David Courson](#), a Physiology course student from the University of Chicago, watching a movie Courson had made of two P granules moving, touching, and combining into one. "We were thinking, man, that looks exactly like two liquid droplets fusing," Brangwynne says, which was completely surprising because P granules, which contain protein and RNA molecules, were thought to be solids, like

kernels. Brangwynne, a physicist who earned his Ph.D. in the lab of soft condensed matter pioneer [Dave Weitz](#), knew that the state of matter (liquid, solid, gel, etc.) has major implications in a cell, so they began making measurements. By the end of the course they were "90 percent sure" that P granules are, in fact, liquid droplets that had separated from the cell's cytoplasm in the same way that oil drops separate from water. This kind of "demixing" of two liquids in live cells had never been seen before, and it immediately suggested a whole new way for cells to organize internally.

[Tim Mitchison](#), then co-directing the Physiology course with [Ron Vale](#), became involved in the exciting discussions of what they might be seeing. "It had always been assumed that P granules get transported to the posterior end of the cell by the actin cytoskeleton [a network of filaments that is a main driver of cellular organization]," Mitchison says. "It turns out that's not true at all. What happens is the P granules dissolve and reform, and on average they dissolve more on the anterior side of the cell and reform more on the posterior side. This was an entirely new way of concentrating molecules at one end of the cell -- by essentially evaporating in one region and condensing in another."

After the course ended, Brangwynne and Hyman continued to study this unexpected phenomenon in worm cells, called liquid-liquid phase separation (LLPS). They published their report in [Science](#) in 2009.

Biologists immediately were excited by the idea that liquid droplets condensing might explain how other cellular structures form that, like P granules, have no membrane to hold their molecules inside. And now, one decade later, it's clear that formation of these droplets,

Photo at top: Like oil in water, the contents of cells can segregate into droplets in a process called phase separation. Oil art: Steve Pavlovsky/Liquid Light Lab originally published in Mar. 14, 2008 issue of *Nature* 555, 300-302.

now often called “biomolecular condensates,” plays a pervasive role in organizing the contents of living cells. Still under study is why cells need to create condensates. They may serve as “reaction crucibles “ that concentrate certain molecules to perform some function; and/or they may sequester molecules not needed for other cellular processes.

Yet the LLPS process “comes with inherent dangers,” [Hyman has written](#). If the protein concentration inside a condensate gets too high and the proteins “jam” or aggregate, the cell may not be able to dissolve the aggregates or limit their growth. Many diseases of the brain are characterized by toxic aggregates of proteins, such as Alzheimer’s, Parkinson’s, and amyotrophic lateral sclerosis (ALS). To date, at least four companies have been founded to investigate the role that LLPS may play in these diseases and others, and hopefully to identify drugs and cures.

But back in 2009, just one example of LLPS in cells had been discovered. And Brangwynne hoped to find more, to demonstrate generality. He loved being at MBL and decided to come back for another summer and “hang out” in Tim Mitchison’s lab, who was returning as a Whitman scientist. There, using frog eggs, Brangwynne started looking at another membrane-free structure found in the nucleus of cells, called the nucleolus. He was able to determine that the nucleolus, too, is a liquid droplet that emerges by phase separation ([Brangwynne, Mitchison and Hyman](#), PNAS, 2011).

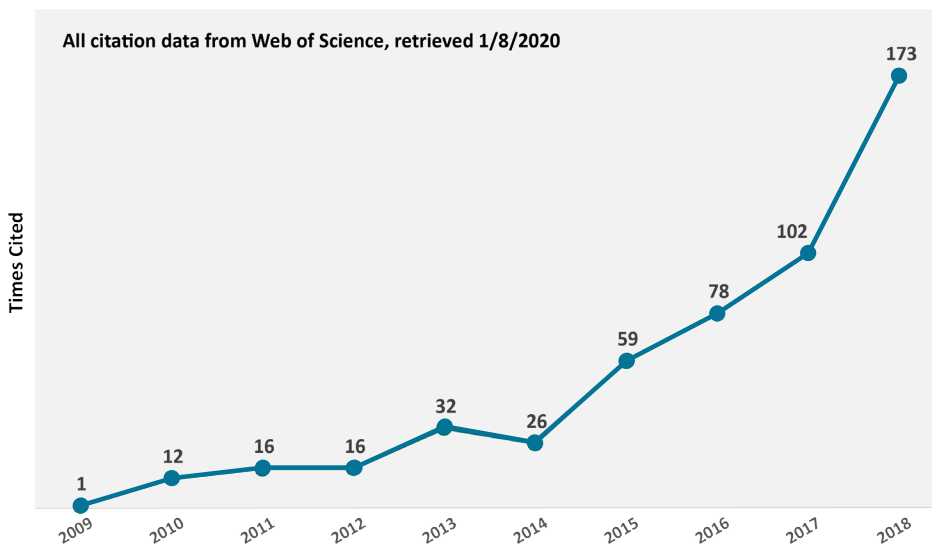
On another front, Ron Vale, now returning to MBL as a Whitman Center scientist, was exploring similar

mysterious, membrane-free assemblies of proteins and/or RNAs. In 2013, Vale teamed up with [Michael Rosen](#), who had published a paper identifying a mechanism for phase separation (Pilong Li et al, Nature, 2011), and [Jim Wilhelm](#) to launch an ambitious “Summer Institute” at the MBL. Funded by Howard Hughes Medical Institute (HHMI), this innovative, cooperative, flexible program convened 70 scientists from around the world over five summers. Collectively, they played a large role in defining and driving the new field of biomolecular phase separation.

[Amy Gladfelter](#), who was part of the Summer Institute, says “it was really, really important for defining the problems in this new field. Our lab meetings were all about grappling with definitions and functions and even what terminology to use. I can’t say enough about how important the Institute was.” Said Rosen of the Institute, “The whole was much more than the sum of the parts. That is one of the wonderful things that MBL provides: a history and a mechanism of [convening] collaborators to work together.”

At least 25 papers came out of the MBL-HHMI Summer Institute, many of fundamental and potentially medical importance in the emerging field. Vale and Ankur Jain, for instance, discovered that nuclear RNA phase transitions may be a key factor in a class of severe neurological and neuromuscular diseases that include Huntington disease, muscular dystrophy, and ALS. (Jain and Vale, [Nature](#), 2017).

The far-reaching implications of LLPS continue to be explored in the MBL courses and in the Whitman Center.



**Citations of Original 2009 Liquid-Liquid Phase Separation Paper**

Liquid-liquid phase separation as an organizing principle in cells is an increasingly significant topic in biological and medical research.

The original 2009 paper by Brangwynne et al in Science, “Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation,” was cited more than 515 times between 2009 and 2018.