DROPLETS IN THE CELLULAR SOUP

TEXT: TIM SCHRÖDER

For decades, few people were interested in the puncta that biologists observed when they examined cells under the microscope. Cliff Brangwynne and Anthony Hyman from the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden were among the first researchers to study these mysterious phenomena in more detail.

Nowadays, the structure of cells is something that children learn about in school. Cells are generally depicted as small bubbles or rectangles with a nucleus and several organelles floating around inside – including the "Golgi apparatus," in which proteins are modified, and the mitochondria, which are the cells' power plants. If the drawings in schoolbooks are to be believed, a cell contains little else.

In reality, however, the cells are full to the brim. It's estimated that there are around five billion protein molecules inside every cell – and these molecules aren't simply floating around. On the contrary, they join together in a fascinating way to form puncta, which appear from time to time and then merge with one another. Sometimes, dozens of these structures form within minutes and then disappear just as quickly. Researchers have been aware of puncta for as long as microscopes have existed. But for a long time, few people were particularly interested in these small, poorly defined blobs. All of that changed when the cellular biologist Anthony Hyman and his colleague Cliff Brangwynne made an astonishing discovery during a physiology course in 2008. The group was examining eggs from the roundworm *Caenorhabditis elegans* under the microscope when Cliff Brangwynne noticed a number of strikingly large structures inside the cells that behaved like oil droplets in water. This was enough to pique the researchers' curiosity!

Since then, a fascination with puncta has spread throughout the scientific community. Today, we know that these structures are actually accumulations of proteins and other large molecules such as nucleic acids – and that they are not formed by chance. On the contrary, their growth and dissolution is actively controlled by the cells themselves. Anthony Hyman, Director at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, refers to these protein structures as "condensates." They are formed when proteins come together in the cellular fluid (cytosol) and form a denser mass. In other words, the proteins transition to a new "phase."

Many scientists now believe that these protein condensates play a vital role in biochemical processes within the cells, whether it be in cell division, in reading the genetic code and producing proteins, or in the development of diseases. "In most cases, we still don't know exactly what drives the formation of condensates or what function the various structures perform," says Hyman. As an example, if you re-

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IMAGE: MOHAMAD ALMEDAWAR/MPI OF MOLECULAR CELL BIOLOGY AND GENETICS

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A fluorescent, saccharolytic enzyme stains endothelial cells violet. If the cells are suffering from a lack of oxygen, certain proteins assemble into aggregates (green spots). These proteins come together with the saccharolytic enzyme in the white spots.

placed the five billion protein molecules with people and kept the density the same, all of those people would be contained within a volume roughly corresponding to the that of Lake Como. The fascinating thing is that the proteins can rearrange themselves within the space of a few seconds and thereby form condensates.

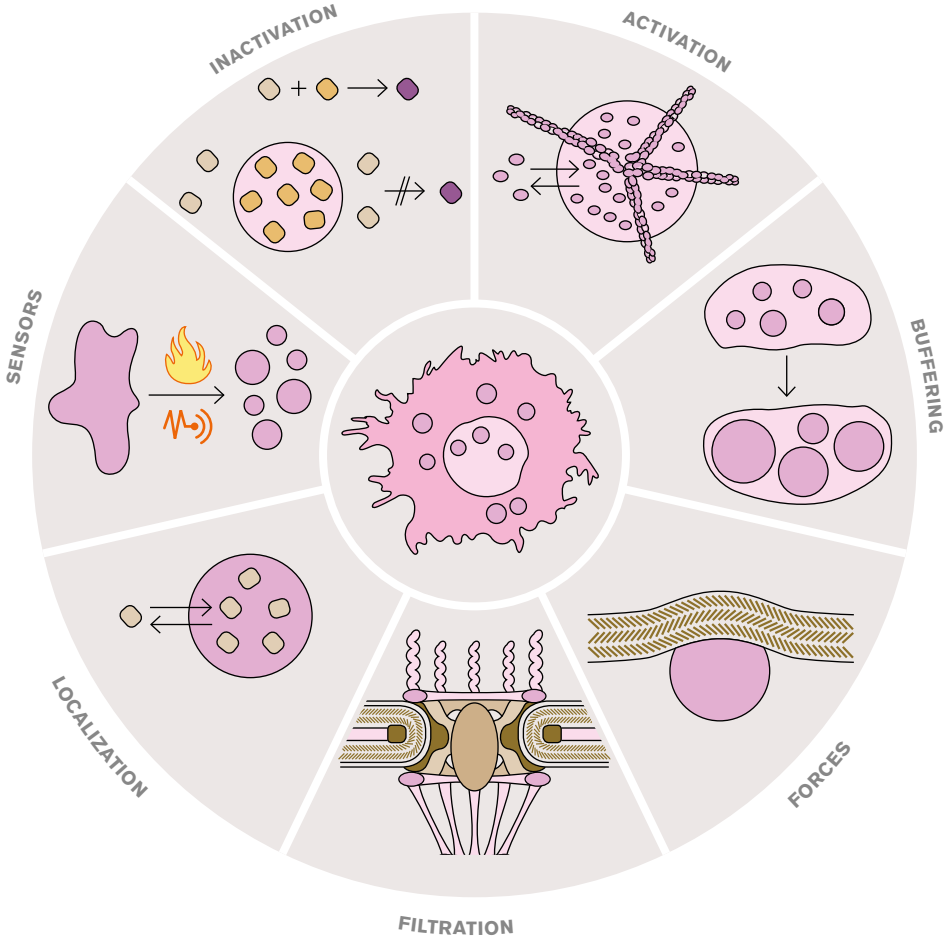
Frightful aggregates

Anthony Hyman and his team are exploring when and how proteins come together in the cells – for example, by investigating the temperatures or salt levels at which the protein condensates form. These are important insights because many diseases probably occur when the natural rhythm of condensate formation and dissolution gets out of sync. One example is the so-called "tau" protein. Inside cells, this protein regulates the assembly of "microtubules" – long, threadlike molecules that form part of the cytoskeleton. If the tau protein adopts an incorrect three-dimensional structure, it produces the deposits – i.e. "plaques" – typically associated with Alzheimer's disease. Another example is amyotrophic lateral sclerosis (ALS), a disease characterized by the death of nerve cells that control the muscles. If these cells fail to do their job, the body can no longer move muscles even though they are otherwise intact. Hyman and his team studied the "fused in sarcoma" (FUS) protein, whose mutated forms are associated with the development of ALS. The FUS protein is normally found in the cell nucleus. In cells that are exposed to environmental stress, however, it leaves the nucleus and forms droplets in the cytosol. When Hyman's group produced droplets using FUS proteins with mutations similar to those in ALS, they made an alarming discovery: after a few hours, the droplets had solidified into "frightful aggregates," as Hyman puts it. Clearly, the mutated proteins had triggered a phase transition from a liquid into a solid, crystal-like state that could also be a cause of the disease.

Nowadays, researchers have a good understanding of the principles underpinning phenomena such as the phase transitions from solid to liquid and the formation of condensates. Physicists have studied these phenomena and described them in detail.

Hari Raj Singh (left) and Sina Wittmann in the laboratory at the Max Planck Institute of Molecular Cell Biology and Genetics. Together with their colleagues, they want to find out how biocondensates form in cells – and what functions they perform.

PHOTO: MPI OF MOLECULAR CELL BIOLOGY AND GENETICS



GRAPHIC: GCO BASED ON CELL. 2019 JAN 24; 176(3)

Scientists still haven't discovered all of the functions that condensates perform in cells. Discussions focus on (clockwise from top): the activation of reactions; the storage of surplus molecules (buffering); the generation of mechanical forces; the modification of membrane pore size (filtration); and the localization of molecules at a specific site. If the condensates only form under certain conditions, they could also be used as sensors. Furthermore, they could prevent reactions between molecules by keeping them separate from one another (inactivation).

Key factors include the concentration of the substances, the electric charges of the proteins involved, and the fact that certain protein sections are more hydrophilic or hydrophobic. That being said, researchers still haven't figured out how cells start and stop the process of phase separation and condensation – or how they control it. In principle, the protein concentration in the cells must be high in order for condensates to form. Anthony Hyman therefore asked himself not only why the puncta form in certain diseases, but also how the process of aggregation is initiated or inhibited in healthy cells. "Why isn't the cell like a scrambled egg, with the proteins clumped together? After all, the proteins in the cytosol are at such a high concentration that they should actually be precipitating out of the solution."

Hyman may now have identified a key molecule in this process, namely adenosine triphosphate (ATP) – the molecule that supplies living cells with almost all of their energy. His ears pricked up when his team added ATP to protein condensates and found that the condensates disappeared. Apparently, ATP prevents the proteins from aggregating, even at the high concentrations inside a cell.

Better solubility in water

ATP appears to be acting like "hydrotropic" substances, which are not solvents themselves but are used in the chemical industry to increase the solubility of organic compounds in water. This molecule occurs in large quantities in cells, and it is conceivable that it only has a hydrotropic effect at certain concentrations. In that case, a change in concentration would affect the solubility of protein condensates. "It's possible that ATP originally developed as a biological hydrotrope in order to keep biomolecules soluble at a high concentration, and that life only began to use it as an energy source at a later stage," explains Hyman. This hypothesis is difficult to prove experimentally, however, because it's almost impossible to modify the hydrotropic properties of ATP without affecting its role as an energy source. "But if this hypothesis is correct, it would explain why protein aggregates often form in age-related diseases – because ATP production decreases with age."

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Protein condensates may also play an important role in cell division. Before a cell divides, the chromosomes arrange themselves in the middle of the cell. Within a few minutes, the cell then forms a rigging-like structure known as a "spindle" – and this apparatus acts like a series of tow ropes, pulling the chromosomes into the two daughter cells. This spindle is also formed by protein condensation and emerges when individual tubulin molecules stack up alongside one another in the cytosol to form long chains known as microtubules. But how exactly are these microtubules formed? Hyman and his team conducted an experiment in which they produced condensates of the microtubule-binding tau protein and added tubulin, which migrated into the tau droplets. As the tubulin was now at a significantly higher concentration inside the tau droplets, it triggered the formation of microtubules. Hyman and his colleagues therefore suspect that cells use phase separation as a way of initiating microtubule growth and cell division.

As yet, Hyman is unable to say how important the condensates will turn out to be in cell physiology as a whole. Though previously thought of as nothing more than blobs inside a cell, these condensates are now known to be structures that form according to specific molecular rules – and experts are steadily gaining a better understanding of how these rules work. "There's now a great deal of evidence to suggest that these are actually biochemical micro-factories." Despite these findings, some researchers still believe the condensates to be irrelevant, while others see them as one of the most important discoveries in modern biology. "It remains to be seen which view is correct – or whether the truth lies somewhere in-between," says Hyman.

Anthony Hyman is considered one of the discoverers of phase separation in cells. When studying eggs of the roundworm *C. elegans,* it occurred to him that the accumulations of RNA molecules inside the cells were behaving like droplets of oil in water.

PHOTO: SVEN DÖRING

SUMMARY

Proteins can form bubble-shaped aggregations inside a cell. These condensates can develop and dissolve again within seconds or minutes.

Condensates are likely to be involved in fundamental processes, such as cell division and protein production.

Scientists suspect that diseases such as Parkinson's or Alzheimer's are caused by the uncontrolled formation of protein condensates in cells.

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