Peering inside cells to see how they respond to stress

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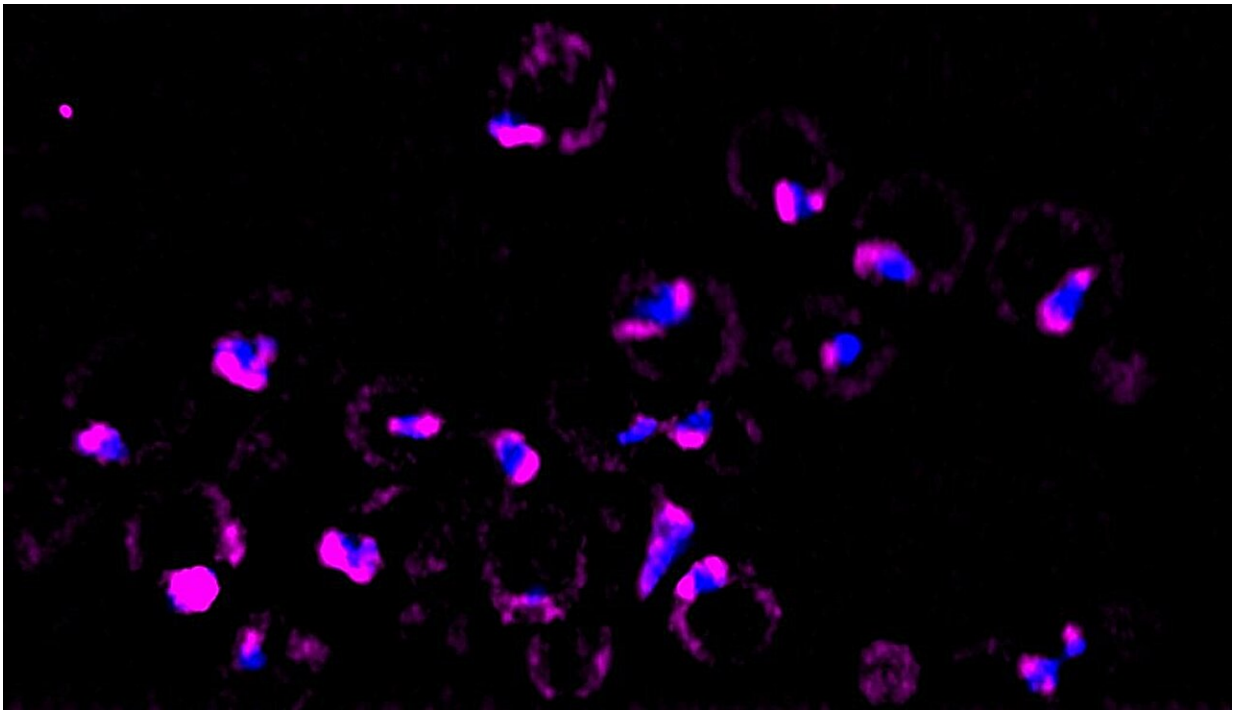

Lattice light sheet microscopy captured the condensation of orphan ribosomal protein (Rpl26_halo, pulse-labeled to trace newly synthesized ones) around the perinucleolar region (marked in blue by Rpa190 nucleolar marker) during a yeast heat shock. Credit: Asif Ali / UChicago

Imagine the life of a yeast cell, floating around the kitchen in a spore that eventually lands on a bowl of grapes. Life is good: food for days, at least until someone notices the rotting fruit and throws them out. But

then the sun shines through a window, the section of the counter where the bowl is sitting heats up, and suddenly life gets uncomfortable for the humble yeast. When temperatures get too high, the cells shut down their normal processes to ride out the stressful conditions and live to feast on grapes on another, cooler day.

This "heat shock response" of cells is a classic model of biological adaptation, part of the fundamental processes of life—conserved in creatures from single-celled yeast to humans—that allow our cells to adjust to changing conditions in their environment.

For years, scientists have focused on how different genes respond to heat stress to understand this survival technique. Now, thanks to the innovative use of advanced imaging techniques, researchers at the University of Chicago are getting an unprecedented look at the inner machinery of cells to see how they respond to heat stress.

"Adaptation is a hidden superpower of the cells," said Asif Ali, Ph.D., a postdoctoral researcher at UChicago who specializes in capturing images of cellular processes. "They don't have to use this superpower all the time, but once they're stuck in a harsh condition, suddenly, there's no way out. So, they employ this as a survival strategy."

Ali works in the lab of David Pincus, Ph.D., Assistant Professor of Molecular Genetics and Cell Biology at UChicago, where their team studies study how cells adapt to stressful and complex environments, including the heat shock response.

In the new study, published October 16, 2023, in *Nature Cell Biology*, they combined several new imaging techniques to show that in response to heat shock, cells employ a protective mechanism for their orphan ribosomal proteins—critical proteins for growth that are highly vulnerable to aggregation when normal cell processing shuts down—by

preserving them within liquid-like condensates.

Once the heat shock subsides, these condensates get dispersed with the help of molecular chaperone proteins, facilitating integration of the orphaned proteins into functional mature ribosomes that can start churning out proteins again. This rapid restart of ribosome production allows the cell to pick back up where it left off without wasting energy.

The study also shows that cells unable to maintain the liquid state of these condensates don't recover as quickly, falling behind by 10 generations while they try to reproduce the lost proteins.

"Asif developed an entirely new cell biological technique that lets us visualize orphaned ribosomal proteins in cells in real time, for the first time," Pincus said. "Like many innovations, it took a technological breakthrough to enable us to see a whole new biology that was invisible to us before but has always been going on in cells that we've been studying for years."

Loosely affiliated biomolecular goo

Ribosomes are crucial machines inside the cytoplasm of all cells that read the genetic instructions on messenger RNA and build chains of amino acids that fold into proteins. Producing ribosomes to perform this process is energy intensive, so under conditions of stress like heat shock, it's one of the first things a cell shuts down to conserve energy.

At any given time though, 50% of newly synthesized proteins inside a cell are ribosomal proteins that haven't been completely translated yet. Up to a million ribosomal proteins are produced per minute in a cell, so if ribosome production shuts down, these millions of proteins could be left floating around unattended, prone to clumping together or folding improperly, which can cause problems down the line.

Instead of focusing on how genes behave during heat shock, Ali and Pincus wanted to look inside the machinery of cells to see what happens to these "orphaned" ribosomal proteins. For this, Ali turned to a new microscopy tool called lattice light sheet 4D imaging that uses multiple sheets of laser light to create fully dimensional images of components inside living cells.

Since he wanted to focus on what was happening to just the orphaned proteins during heat shock, Ali also used a classic technique called "pulse labeling" with a modern twist: a special dye called a "HaloTag" to flag the newly synthesized orphan proteins.

Often when scientists want to track the activity of a protein inside a cell, they use a green fluorescent protein (GFP) tag that glows bright green under a microscope. But since there are so many mature ribosomal proteins in a cell, using GFPs would just light up the whole cell. Instead, the pulse labeling with HaloTag dye allows researchers to light up just the newly created ribosomes and leave the mature ones dark.

Using these combined imaging tools, the researchers saw that the orphaned proteins were collected into liquid-like droplets of material near the nucleolus (Pincus used the scientific term "loosely affiliated biomolecular goo"). These blobs were accompanied by molecular chaperones, proteins that usually assist the ribosomal production process by helping fold new proteins. In this case, the chaperones seemed to be "stirring" the collected proteins, keeping them in a liquid state and preventing them from clumping together.

This finding is intriguing, Pincus said, because many human diseases like cancer and neurodegenerative disorders are linked to misfolded or aggregated clumps of proteins. Once proteins get tangled together, they stay that way too, so this "stirring" mechanism seems to be another adaptation.

"I think a very plausible general definition for cellular health and disease is if things are liquid and moving around, you are in a healthy state, once things start to clog up and form these aggregates, that's pathology," Pincus said. "We really think we're uncovering the fundamental mechanisms that might be clinically relevant, or at least, at the mechanistic heart of so many human diseases."

Finding structure at an atomic scale

In the future, Ali hopes to employ another imaging technique called cryo-electron tomography, an application using an electron microscope while cell samples are frozen to capture images of their interior components at an atomic level of resolution. Another advantage of this technique is that it allows researchers to capture 3D images inside the cell itself, as opposed to separating and preparing proteins for imaging.

Using this new tool, the researchers want to peer inside the protein condensates to see if they are organized in a way that helps them easily disperse and resume activity once the heat shock subsides.

"I have to believe they're not just jumbled up and mixed together," Pincus said. "What we're hoping to see within what looks like a disorganized jumbled soup, there's going to be some structure and order that helps them start regrowing so quickly."

More information: Adaptive preservation of orphan ribosomal proteins in chaperone-dispersed condensates, *Nature Cell Biology* (2023). DOI: 10.1038/s41556-023-01253-2

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