Protein aggregation after heat shock is an organized, reversible cellular response

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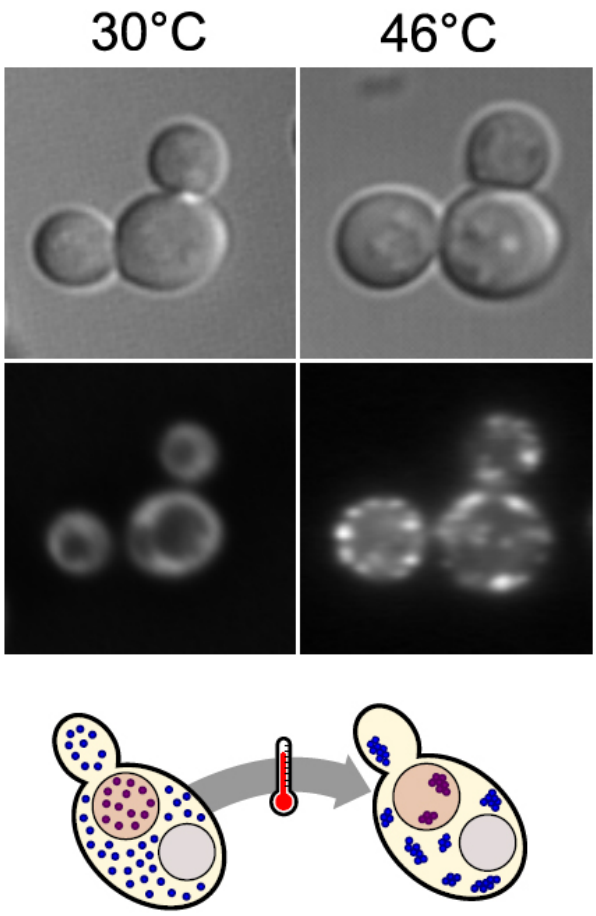

Microscope images of yeast cells before and heat shock. Fluorescent labeled heat-shock-granule-forming protein (Ola1) marks protein aggregates (bottom right). Credit: D. Allan Drummond

Protein aggregates that form after a cell is exposed to high, non-lethal temperatures appear to be part of an organized response to stress, and not the accumulation of damaged proteins en route to destruction. Reporting in *Cell* on Sept. 10, 2015, scientists from the University of Chicago and Harvard University discovered that aggregates are fully reversible - after the cell returns to normal temperatures, aggregated proteins are disentangled and resume their normal cellular functions. Some proteins were found to remain intact and even functional while in an aggregated state.

The findings shed new light on the biological nature of protein aggregates, which have been widely considered to be toxic dead-end products, but are increasingly being recognized as a new layer of cellular organization.

"We asked simple questions: what proteins aggregate in the cell during acute heat shock and what happens to them when the cell recovers?" said senior study author D. Allan Drummond, PhD, assistant professor of biochemistry and molecular biology at the University of Chicago. "Much to our surprise, we found that even the most severely aggregated proteins disassembled and went back into circulation during recovery. It raises the possibility that most of these aggregates, which before looked like damage, may actually be part of a coordinated, evolved process."

Despite decades of investigation, many questions remain about how cells respond to heat shock. When exposed to stressful, but nonlethal temperatures, some cellular proteins aggregate into large clumps known as stress granules. Mutant cell line studies have suggested that certain types of stress granules are destroyed by the cell and their protein components presumably remade. Artificially-introduced heat-sensitive proteins, as well as newly made proteins, have been shown to be aggregation-prone and destroyed by cells after heat shock. But whether similar fates befell the vast majority of proteins in normal cells remained unclear.

To look at the effects of heat shock on native populations of proteins, Drummond and his colleagues utilized a novel set of techniques that allowed them to simultaneously track almost 1,000

different mature proteins in yeast cells. The team exposed cells to temperatures ranging from 30°C (normal) to 46°C (severe heat shock), for very short periods of time, from two to eight minutes. They then measured protein aggregation with tools that included mass spectrometry.

The researchers identified more than 175 different proteins that aggregated in response to heat shock, representing around a sixth of the proteins measured and about ten times as many as were known before. Specific proteins formed granules at specific and separate cellular locations, indicating a level of organization. However, the team also found that aggregation happened under many conditions where stress granules did not form, indicating that aggregation and formation of granules are related but separate processes.

The biggest surprise came when the team looked at the fates of aggregated proteins. Proteins were labeled with isotopes—a technique similar to carbon dating for archaeological finds—and followed as cells underwent heat shock and recovery. They found that aggregated proteins were disentangled and resumed their original functions without exception after cells returned to normal temperatures. Isotope labeling ruled out the possibility that aggregated proteins were being degraded and replaced by new proteins, which would be unlabeled.

The team then performed a detailed analysis on three proteins of interest, which in normal conditions form a complex that links amino acids to transfer RNAs (tRNAs). When isolated and tested for their response to heat, these proteins readily aggregated. But even in an aggregated state after severe heat shock, they formed a functional complex that still actively and accurately processed tRNAs.

"In contrast to what's been observed in many studies on foreign proteins and mutant cells, disassembly of native aggregates after heat shock in normal cells is essentially complete under these conditions," Drummond said. "Aggregation likely inactivates the vast majority of proteins, but it's remarkable that some can remain active when aggregated. All of this indicates the need to rethink the biological meaning of aggregation during heat shock."

Based on previous work and their own results, Drummond and his colleagues speculate that a central purpose of protein aggregation during heat shock is to reshape the cellular factory, focusing protein synthesis on proteins needed during stress. In some cases, the authors hypothesize, proteins act as autonomous thermometers and actuators, sensing heat and self-assembling to activate or deactivate certain cellular functions.

"Heat-induced aggregation has all the hallmarks of an adaptive response," said study author Edward Wallace, PhD, postdoctoral scholar in biochemistry and molecular biology at the University of Chicago. "Our findings suggest a layer of cellular machinery that senses and enacts these decisions by forming specific aggregates at specific places and times."

The findings also raise intriguing questions about the nature of protein aggregates, which are seen in a wide range of neurodegenerative diseases. The team are now working to better understand the biological functions of the aggregates, particularly in their roles as regulators of cell function. They have also started harnessing the temperature-sensing parts of proteins for biotechnological applications, like separating one type of molecule from another in response to a shift in temperature.

"By taking a careful, modern look at an old problem, we got surprising results that change the way we think not just about heat shock, but about how cells sense and respond to their environment at the molecular level," Drummond said. "This is what's so rewarding about studying the biology of basic processes."

More information: "Reversible, Specific, Active Aggregates of Endogenous Proteins Assemble upon Heat Stress," *Cell*, 2015.

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