Investigation of intracellular temperature during stress granule formation

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Temperature, a fundamental physical parameter, governs a wide variety of intracellular chemical reactions and biological processes. In our previous research, we have developed methods to track and image the intracellular temperature with a fluorescent polymeric thermometer (FPT) and fluorescence microscopy. By using these methods, we discovered that the intracellular temperature was different between each organelle and changed in response to stimulations within a single living cell. Furthermore, we found that the local temperature near the mitochondria increased upon stimulation. These results indicated that local intracellular temperature might be intrinsically related to fundamental cellular processes. However, the detail and significance of intracellular temperature are still unclear. In this study, we focused on the intracellular temperature during stress. In eukaryotic cells, cytoplasmic mRNAs assemble and form stress granule (SG) during a stress response induced with a variety of stress conditions such as arsenite oxidative stress and heat-shock. SG serves as a key modulator of post-transcriptional and epigenetic gene expression. According to recent researches, it is supposed that mitochondria might play an important role in the process of SG formation. Therefore, we hypothesized that the intracellular temperature change concerning mitochondria under stress might function in cell signaling.

To confirm our hypothesis, we here performed intracellular temperature measurement in living COS7 cells by using FPT during SG formation. We also developed an Infrared-laser local heating system to manipulate the local temperature in living cells.

The results showed that the intracellular temperature increased under the condition of arsenite stress. Next, we investigated the relationship between intracellular thermogenesis and SG formation. To provoke thermogenesis in mitochondria, we introduced FCCP (Carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone), an uncoupling agent, into COS7 cells. As the concentration of FCCP increased, intracellular temperature elevated, which allowed mRNA aggregate to form SG in a dose-dependent manner. We also successfully observed SG formation induced by IR laser local heating system as an artificial thermogenesis. These results demonstrated that SG formation have intrinsic relationship with local intracellular temperature change, which may be a novel mechanism of this phenomenon. Thus, our research might reveal a deeper understanding of intracellular temperature and cellular functions.