Probing ER-related Stress with Lipid Markers

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Robust stress response pathways are essential for survival and fitness in all cells. Here we investigate a, to the best of our knowledge, previously uncharacterized stress response in budding yeast. Specifically, we observed an unexpected high-intensity, intermittent fluorescence signal indicative of stress response in *S. cerevisiae* grown in the presence of fluorescently-conjugated lipids. Using single cell microscopy-based analysis, we determined that this signal originates from the endoplasmic reticulum (ER), and is related to changes in membrane potential. Here we quantify this phenomenon with the aim to characterize a novel mechanism for responding to ER-associated stress.

I. Introduction

STRESS response pathways enable life to persist in dynamic environments. An important class of stress responses control and maintain the homeostasis of cells' cytoplasm and organelles, e.g. by controlling the intracellular osmolarity [1]. While responses to such stresses may be short-lived, manifest as severe changes in physiology and morphology, and even fire irregularly, failure to respond can be fatal. Here, we develop new methods to characterize stress-responses using lipid probes, and use our new methods to investigate what might be a novel ER-mediated stress response.

II. Investigation

Fluorescently-conjugated lipids are used as reporters in many different contexts and can be used to gain insight into the function and composition of lipid membranes, including in *S. cerevisiae*. Here we investigate the effects of fluorescently-conjugated lipids on yeast cells grown in its presence. Surprisingly, we noted that cells grown in the presence of the fluorescently-conjugated lipids display intermittent bursts of short-lived, high intensity fluorescence. Importantly, a minor fraction of come populations of cells remained in a high fluorescent state and died, as is characteristic of a population enduring a stress response.

Next, we determined the subcellular localization of the phenomenon. Colocalization of the signal from the fluorescently-conjugated lipids with a signal from fluorescently-tagged Erg6, which served as a marker for the ER, clearly demonstrates that the stress and/or stress-response are associated with the ER. This result also suggests that the ER membrane, in which the fluorescently-conjugated lipids may be incorporated, undergoes perturbations during this stress event.

Interestingly, we also observed that the intermittent fluorescent signal was sometimes accompanied by a decrease in cell volume. Rapid perturbations of cell volume in yeast are normally seen when cells respond to changes in the extracellular osmolarity, a response mediated by the high-osmolarity glycerol (HOG) pathway. To test if the HOG pathway also governs the observed bursts of fluorescence, we tagged Hog1 with a fluorescent reporter and observed its dynamics while cells were exposed to fluorescently-conjugated lipids. Surprisingly, we noted that the HOG pathway did not appear to be induced in cells displaying the bursting phenotype. This suggests that we are observing a novel stress response.

Since the observed fluorescence phenomenon may be related to cell volume changes and is localized to the ER membranes, we next investigated whether changes in membrane potential are related to the bursting phenotype. We found that it was possible to modulate the frequency of the intermittent blinking signal via incorporation of different ions in the media, indicating that the fluorescence phenomenon is indeed related to changes in membrane potential.

III. Future Directions

To understand the mechanism that controls this phenomenon, we will monitor major signaling/stress response pathways (including PKA, TORC1, etc.) to determine which pathway(s) control this phenomenon. Here, we will capitalize on our recently developed system of fluorescently-tagged proteins that allows us to tag and monitor up to six fluorophores at the same time, allowing us to simultaneously monitor multiple pathways in single cells. By quantifying this signal and its relationship with changes in membrane potential and signaling, we will determine whether this is indeed a *bona fide* new stress response in yeast. Moreover, we will characterize the spatial and temporal organization of important stress response pathways surrounding an ER-associated event and describe novel methods for interrogating stress response at the single cell level.

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