Arabidopsis Heat Shock Granules exhibit dynamic cellular behavior and can form in response to protein misfolding in the absence of elevated temperatures

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Abstract

Figure 1. Heat shock granule dynamics in Arabidopsis roots.: For A-H cell walls were stained with propidium iodide and are shown in magenta. A, B) BOB1:GFP distribution under control (22°C) conditions. C, D) BOB1:GFP distribution after a 2 hour 37°C heat shock. E, F) BOB1:GFP distribution after a 4 hour incubation in 0 mM amino acid analog L-Azetidine 2-Carboxylic Acid (AZC) at 22°C. G, H) BOB1:GFP distribution after a 4 hour incubation in 10 mM AZC at 22°C. Arrows highlight heat shock granules (HSGs). I) Maximal intensity projection of BOB1:GFP in a root tip after a 1
We observed variations in the size and number of HSGs depending on the HS regimen used in our experiments. Longer periods of HS appeared to result in fewer and larger granules. To the best of our knowledge, no quantitative characterization of plant HSG dynamics has been published although a similar observation has been made using eIF4A2:GFP labeled SGs (Hamada et al., 2018). We set out to ask how HSG size and number change with increasing durations of HS. In order to quantify these differences we used 3D confocal imaging to quantitate HSG number and size during an extended 37°C HS. Over the course of the HS the number of HSGs decreased while their volume increased (chi-squared p<10^{-175}) (Fig. 1 I-K).

Several mechanisms could explain this result. It is possible that during an extended HS a subset of HSGs grow larger while others disassemble. The oligomerization state of sHSPs is known to be dynamic and so perhaps HSGs compete with each other for misfolded protein/chaperone complexes. Alternatively, HSGs could grow over time by fusing with each other. To distinguish between these possibilities we used 4D confocal imaging to observe HSG dynamics every 30 seconds over extended periods of time (>30 minutes). We did not detect heterogeneity in HSG behavior. Rather, we repeatedly observed HSG fusion events when HSGs came into contact with each other (Fig. 1 L and Movie 1). These results suggest...
that during an extended HS, as misfolded proteins accumulate in the cytoplasm and start to require chaperone function, HSGs form. As the load of misfolded protein increases with extended proteostatic stress, HSGs grow larger, presumably due to the incorporation of more misfolded proteins, but also by fusing with each other.

We have demonstrated that BOB1:GFP can be used to visualize HSG formation caused by two different cellular stresses: HS and AZC, and that prolonged heat stress results in a decrease in the number of HSGs while their size increases. The AZC results suggest that the function of HSGs is to protect plant cells from protein misfolding, regardless of the cause of the denaturation. The ability to quantitate HSG size and number in vivo using BOB1:GFP provides a protein based marker for visualizing misfolded cytoplasmic protein accumulation in plants.

**Methods**

**Plant growth and chemical treatment**

For still imaging, BOB1:GFP seeds (Perez et al., 2009) were surface-sterilized, plated on 0.5X MS media, and grown under 16hr light/8hr dark conditions at 22°C. For heat shock treatments, plates were transferred to a 37°C incubator for the indicated times. Plates containing control plants were left in the 22°C incubator. For L-Azetidine-2-Carboxylic Acid (AZC) treatments, whole three-day-old seedlings grown as above were immersed in 0.5X MS media containing 10 mM AZC (BAChem, Torrence, CA). Control plants were immersed in 0.5X MS media. Both control and AZC treated plants were incubated in liquid treatment for four hours in a 22°C incubator with constant light.

For live heat shock time-lapse visualization, BOB1:GFP seeds were germinated in chambered coverslips (Lab-Tek, Thermo Fisher Scientific, Rochester, NY, USA) containing 4 mL 0.5X MS media and 0.5% agarose (Benchmark Scientific, Edison, NJ, USA). Coverslides were tilted 60° after seedling roots had penetrated the MS/agar layer to ensure roots would grow parallel to the coverslip. Surface-sterilized seeds were grown at 22°C in 16hr light/18hr dark conditions for 5 or 6 days.

**Imaging**

For still imaging, three day old seedlings were stained with 10 µg/mL propidium iodide to allow visualization of cell walls. Confocal imaging was performed using a Leica SP5 AOBS confocal microscope (Leica Microsystems) fitted with a heated stage (Tokai Hit, Model INUB-GSI-F1, Shizuoka-ken, Japan) to allow real-time visualization of samples during heat shock. Granule detection and quantitation was performed using Imaris 7.1 software (Bitplane Scientific, South Windsor, CT, USA). Briefly, the surfaces module of Imaris was used to automatically detect three-dimensional HSG surfaces which contained spheres of maximum diameter 0.5 µm. After automatic detection, detected surfaces were filtered based on satisfying minimum intensity threshold values. Appropriate threshold values were determined and edited by visual inspection of representative particles. Detected granule surfaces were filtered such that the minimum enclosed volume was 0.5 µm³.

Time-lapse series were collected for individual plant roots subjected to a continuous 37° heat shock treatment. Z-stack images of 760 X x 352 Y pixels (70 x 34 µm) with 7 Z focal planes separated by 1 µm were collected every 10 s for 50 consecutive frames (8.3 minutes).

**Reagents**

BOB1:GFP seeds are available from the ABRC (stock number CS69998) and from NASC (stock number N69998)

**References**


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