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Erin J. Kast, '15

Nicholas J. Kaplinsky
Swarthmore College, nkaplin1@swarthmore.edu

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**HSFA1d regulates the kinetics of heat-induced HSP17.6 expression in Arabidopsis**

Erin J Kast¹, Nick Kaplinsky¹§

¹Department of Biology, Swarthmore College

§To whom correspondence should be addressed: nkaplin1@swarthmore.edu

**Abstract**

Arabidopsis contains four HSFA1 heat shock transcription factors (HSFA1a, HSFA1b, HSFA1d, and HSFA1e) that regulate the primary response to high temperature stress responses. These genes have overlapping functions and, while double and triple HSFA1 mutants have thermotolerance phenotypes, these genes have no reported single mutant thermotolerance phenotypes. We used an automated fluorescence microscopy system to quantitate the expression of a HSP17.6:GFP reporter with high temporal resolution to show that HSFA1d is required for normal heat-induced HSP17.6 expression. HSP17.6 expression is reduced and delayed in hsfa1d-1 mutants. This finding highlights the power of using gene expression kinetics as a quantitative phenotype for discovering the function of genes that exhibit functional redundancy.

**Figure 1.**

HSP17.6p:GFP heat shock kinetics in wild type and hsfa1d-1 seedlings. Four day old seedlings were heat shocked for one hour at 37°C starting at time point 0. They were grown for an additional 11 hours at 22°C after the heat shock and images of their roots were acquired every four minutes both during and after the heat shock. Wild type plants (n=11) are shown in blue, hsfa1d-1 plants (n=49) in orange. Hsp17.6:GFP expression is measured in arbitrary units. Error bars are +/- S.D.

**Description**

In plants, phenotyping the heat shock response (HSR) is often performed using thermotolerance assays based on survival or germination or by measuring RNA or protein levels at a single or a small number of time points (Yeh et al., 2012; Silva-Correia et al., 2014). These approaches have a limited ability to detect HSR phenotypes in genes with quantitative effects such as members of gene families with partially overlapping functions. An example of this difficulty is illustrated by the HSFA1 family in Arabidopsis which consists of four genes (HSFA1a, HSFA1b, HSFA1d, and HSFA1e). To uncover HSR phenotypes due to a loss of HSFA1 function, at least three family members have to be knocked out as mutations in single or two HSFA1 genes have no phenotypes in thermotolerance assays (Liu et al., 2011; Yoshida et al., 2011). Decreased mRNA levels of heat
shock protein were documented in hsf1a1a/hsf1a1b double mutants, demonstrating that assaying gene expression can be used to overcome the sensitivity limitations of thermotolerance assays (Busch et al., 2005).

To overcome the limitations of conventional thermotolerance assays we built a system which would allow us to measure the HSR with high spatial and temporal resolution in large numbers of plants simultaneously. The system consists of an Arabidopsis reporter line containing the HSP17.6 promoter driving a fast-folding GFP. HSP17.6 (AT1G53540) encodes a small heat shock protein that is strongly induced by elevated temperatures and whose expression serves as a proxy for the HSR. The reporter line is imaged using a high-throughput automated fluorescence microscope that has a temperature controlled plant growth chamber (the RootScope), allowing 150 or more seedlings to be subjected to a heat shock while being imaged every four minutes (Kast et al., 2013).

We used the RootScope to investigate whether we could detect HSR expression phenotypes in a HSFA1d (AT1G32330) T-DNA mutant that does not have a thermotolerance phenotype in the absence of other mutations. The hsf1a1d-1 T-DNA line (SALK_022404) is a RNA null (Liu et al., 2011). Wild-type HSP17.6:GFP seedlings and hsf1a1d-1 mutants crossed into the reporter background were imaged during a one hour 37°C heat shock and for 11 hours after the heat shock. Induction of HSP17.6:GFP expression was observed soon after the heat shock ended in both lines. Two differences became apparent during the recovery phase. First, the maximum HSP17.6:GFP expression level was lower in hsf1a1d-1 mutants than in wild type plants (1235±98 AU WT, 756±73 AU hsf1a1d-1, t-test p-value<0.0001). Second, the time from the end of the heat shock until the expression peak was longer in hsf1a1d-1 mutants compared to wild type plants (220±36 minutes WT, 259±29 minutes hsf1a1d-1, t-test p-value=0.0003) (Figure 1). The slower and smaller HSR in hsf1a1d-1 single mutants is consistent with previously established roles for HSFA1 genes as positive regulators of the HSR (Liu et al., 2011; Yoshida et al., 2011). Many Arabidopsis studies investigating temperature responses measure changes in gene expression immediately after a heat shock (Busch et al., 2005; Perez et al., 2009; Liu et al., 2011). This approach would not uncover a phenotype in the case of hsf1a1d-1. The ability to uncover a gene expression kinetics phenotype highlights the power of measuring changes in gene expression over time as an approach for uncovering subtle quantitative phenotypes.

Methods
The SALK_022404 T-DNA allele of HSFA1d was crossed to the previously described HSP17.6:GFP reporter line (Kast et al., 2013). F1 progeny were allowed to self-fertilize and F2 plants were screened for individuals homozygous for both the T-DNA insertion in HSFA1d and the HSP17.6:GFP reporter. The HSFA1d insertion was genotyped as described in Liu et al., 2011. The presence of two copies of the reporter was determined by screening F3 seeds using the RFP seed marker associated with the reporter which is in a pFAST-R07 backbone (Shimada et al., 2010; Kast et al., 2013). Families homozygous for the insertion and the reporter were assayed using the RootScope as previously described (Kast et al., 2013). Briefly, seeds were surface sterilized, plated on 0.5x MS media, and stratified at 4°C for 48 hours. After stratification plates containing seedlings were transferred to E-30B growth chambers (Percival Scientific) and grown for four days at 22°C under constant light conditions. The four day old seedlings were transferred to the RootScope’s temperature controlled imaging chamber and exposed to a one hour 37°C heat shock followed by an 11 hour 22°C recovery while on the microscope. HSP17.6:GFP fluorescence was measured in each plant root tip every four minutes.

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