Researching Chloroplast and Mitochondrial Small Heat Shock Proteins in *Arabidopsis thaliana*

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Abstract

Small heat shock proteins (sHsps) are a type of molecular chaperone ubiquitous across all domains of life. Unlike most chaperones, sHsps act independently of ATP-hydrolysis and are thought to make up the first line of stress response in organisms. Despite their widespread presence in life, the role of sHsps have yet to be fully characterized. I present data on Hsp25.3, a chloroplast localized sHsp in *Arabidopsis thaliana*. My work includes generating a knockout mutant of Hsp25.3 using CRISPR/Cas9 in the Columbia background (the most in-depth studied *A. thaliana* background) as well as data on a Hsp25.3-GFP fusion line, which is part of an ongoing effort to confirm localization of other chloroplast and mitochondrial localized sHsps. *A. thaliana* plants have been confirmed to be transgenic with the Cas9 gene but Hsp25.3 mutations have yet to be identified. Preliminary data of immunoblots of the Hsp25.3-GFP fusion lines indicate that the fusion might not be properly processed and thus does not localize correctly, an observation that could complicate attempts of confirming localization of other sHsps using GFP fusion lines.

Introduction

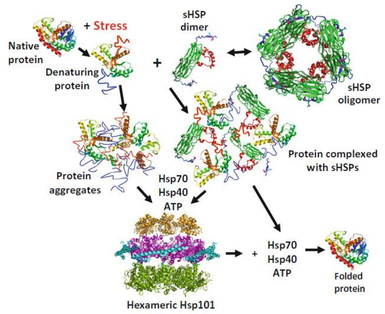

Fig 1. Model of sHsp function. In their native state, sHsps often occur as dimers that oligomerize in their inactive state. In their active state sHsp dimers bind denaturing proteins in an ATP-independent manner and prevent further unfolding and aggregation of their substrate. The sHsp-substrate complex is then targeted to the ATP dependent Hsp70 chaperone (and co-chaperones) for refolding of protein substrate.

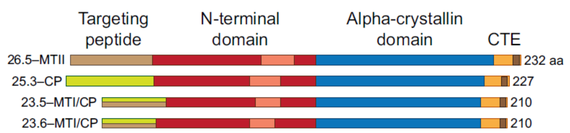

Fig 2. Diversity of organelle and mitochondrial sHsps.
sHsps contain three conserved regions, the N-terminal domain, alpha-crystallin domain and C-terminal extension. Hsp 26.5 is known to localize to the mitochondria, Hsp25.3 localizes to the chloroplast and Hsps 23.5 and 23.6 are thought to dual localize to both chloroplasts and mitochondria.

Overview of my projects:

- Localization of Hsp23.5 + 23.6 not fully confirmed
- GFP fusion lines of all 4 sHsps to test localization under confocal microscopy
- Test sHsp-GFP fusions for proper processing + localization before testing Hsp23.5 + 23.6 localization

Hypothesis:
sHsp-GFP fusions are not properly processed and thus retain their targeting peptide and are not localized properly.

- A second project focuses on generating a new Hsp25.3 knockout mutant in the Columbia background of *A. thaliana* using CRISPR-Cas9.

Fig. 3 Schematic of the Hsp25.3-GFP fusion. The GFP variant mGFP6 was fused C-terminally to the Hsp25.3 sequence (without a stop codon) under the native promoter of Hsp25.3.

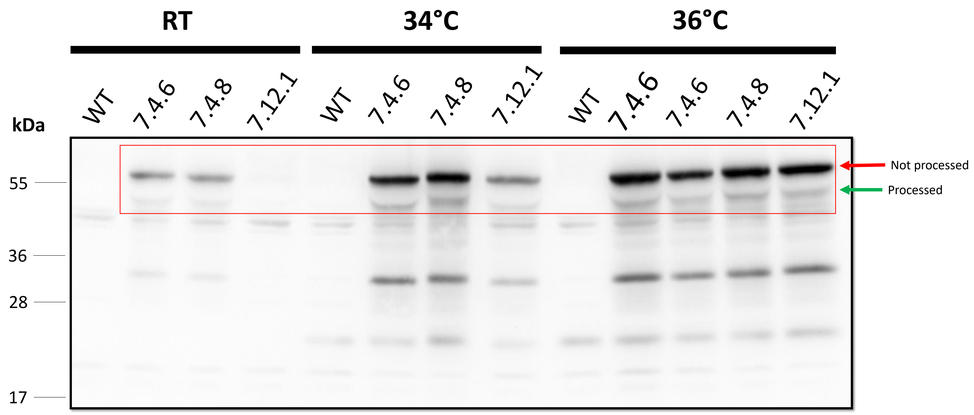

Fig 4. Immunoblots against Hsp25.3 in WT and Hsp25.3-GFP fusion lines.
Probing homozygous Hsp25.3-GFP lines against both Hsp25.3 (and GFP, not shown) indicate that the major species of the Hsp25.3-GFP fusion retains its transit peptide.

Fig 5. Schematic of the Cas9 targets in the Hsp25.3 gene.
Two guide RNAs were designed to target Cas9 to the Hsp25.3 gene resulting in a 488 bp deletion.

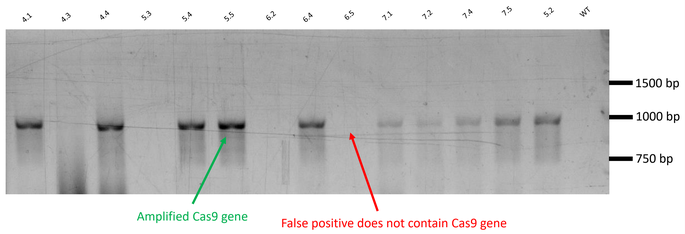

Fig. 7. Most transgenic candidates posses Cas9. PCR amplification of a 936 bp fragment of the Cas9 gene in Hygromycin B resistant seedlings shows that most seedlings are transgenic and are thus expected to be mutated in Hsp25.3

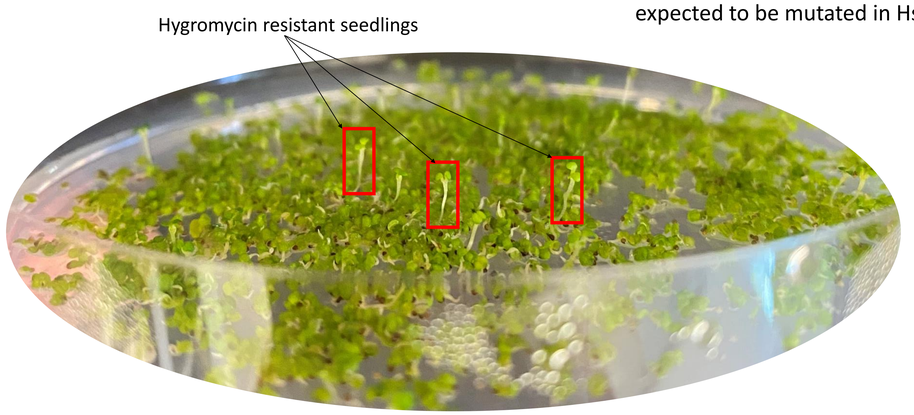

Fig. 6. A small number of seedlings show Hygromycin B resistance.
Hygromycin resistant seedlings are characterized by elongated hypocotyls and longer roots than wild-type seedlings after dark germination.

Discussion + Future Directions

- Hsp25.3-GFP fusion shows that both processed and unprocessed protein is present in the fusion lines
- Fusion expressed even at RT, which is abnormal for sHsps
- Need to obtain clear and representative anti GFP blot
- Test Hsp25.3-GFP in qko line of chloroplast + mitochondrial sHsps
- Test Hsp23.5/23.6 GFP fusions
- If GFP fusions inviable for microscopy: isolate chloroplasts and test for Hsp23.5/23.6

Discussion + Future directions

- Cas9 presence confirms that transgenic candidates are not false positives
- Genotyping of T1 generation was inconclusive
- Test T2 generation for 3:1 segregation
- Genotyping T2 generation and sequence DNA to identify Hsp25.3 mutants

References

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