Investigating Subcellular Localization of *Arabidopsis thaliana* Hydroperoxide Lyase I (At4g15440)

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Introduction

It is widely believed that chloroplasts originated from endosymbiotic bacteria, which gradually transformed into organelles[2]. In this process, many chloroplast genes migrated to the host cell's nuclear genome[5]. The gene products evolved localization signals to help transport them to their destined regions of the chloroplasts[2]. To localize to the chloroplast membrane, proteins may be signal anchored, tail anchored, β – barrel proteins, or contain a cleavable N-terminal transit peptide or reverse transit peptide-like C-terminal sequence[1,3], which are represented in **Figure 1**. Hydroperoxide lyase I (HPL1) is an *Arabidopsis thaliana* chloroplast outer envelope protein involved in stress response by regulating stress response molecules called arabidopsides[4]. While HPL1 is known to be a multi-pass alpha helical transmembrane protein with neither an N-transit peptide nor a C-transit peptide-like sequence[1], it is unknown which regions of HPL1 are responsible for its localization to the chloroplast outer membrane. The objective of this experiment was to determine regions of HPL1 involved in localizing to the chloroplast outer membrane. By elucidating mechanisms of chloroplast protein transport, this study will improve our understanding of chloroplast evolution and organelle biogenesis.

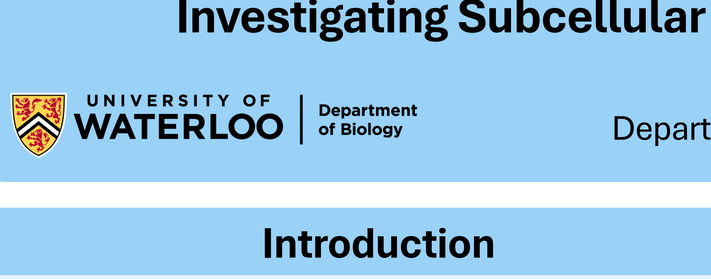

Figure 1: Cartoon diagram of the five known methods of chloroplast membrane targeting. (1) A protein with a cleavable N-terminal transit peptide. (2) β – barrel proteins do not have α-helical transmembrane domain. Instead, they consist of 8-12 strands at a 45° angle from the membrane[1]. (3) Tail anchored proteins are anchored in the chloroplast membrane by a C-terminal α-helix[3]. (4) Signal anchored proteins are anchored in the chloroplast membrane by an approximately 20 amino acid N-terminal α-helix[1]. (5) A protein with a reverse TP-like C-terminal sequence.

Materials and Methods

- Bioinformatic tools were used to predict secondary and 3D structures
- The HPL1 open reading frame was ligated into plasmid vectors containing enhanced green fluorescent protein (EGFP)
- Onion epidermal cells were transformed with the plasmid vectors via biolistic bombardment
- The expression of EGFP in the onion epidermal cells was observed by fluorescent microscopy

Bioinformatic Analysis

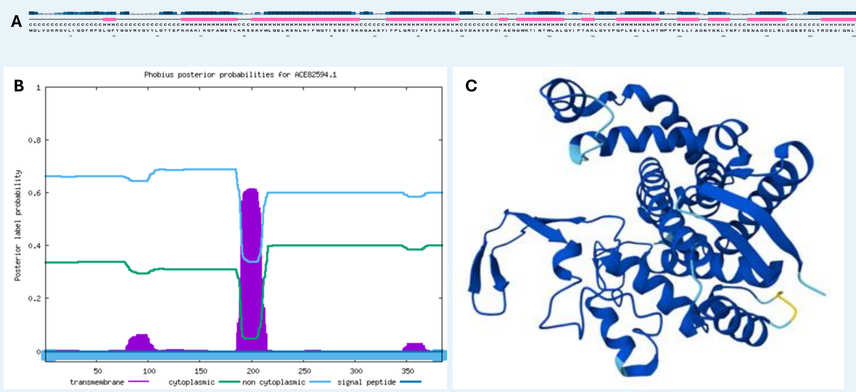

Figure 2: Bioinformatics analyses of HPL1. (A) PSIPRED chart of secondary structure predictions for HPL1 amino acid sequence. Pink regions predict a helix, yellow regions predict a strand, and grey regions predict a coil. No regions of the protein were predicted to be a signal peptide, transmembrane helix, or membrane interaction region. **(B)** Phobius plot of predicted transmembrane, cytoplasmic, non-cytoplasmic, and signal peptide regions in HPL1. Putative transmembrane domains were predicted between about 75-110 aa, 180-220 aa, and 350-370 aa. **(C)** Alphafold 3D structure prediction showed HPL1 as a globular protein composed mainly of alpha helices. Dark blue coloring indicates very high model confidence, light blue indicates high confidence, and yellow indicates low confidence.

Subcellular Localization of HPL1

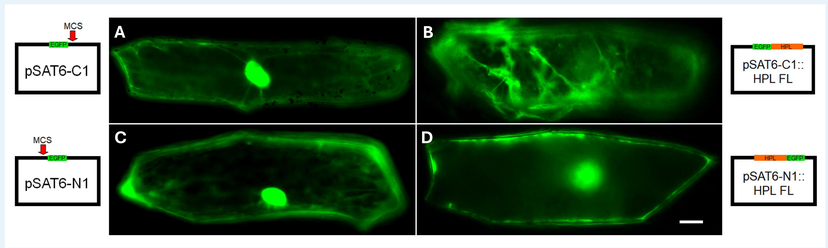

Figure 3: Onion epidermal cells expressing EGFP fusion constructs. (A, C) Empty pSAT6-C1 and –N1 vectors were bombarded into onion epidermal cells as negative controls. The cells showed nuclear and cytosolic EGFP signals. **(B, D)** Onion epidermal cells expressing the full length HPL1 construct showed some localization of EGFP to punctate structures, the nucleus, and cytoplasm. Scale bar = 30 μm

Conclusions and Future Directions

- Bioinformatics analyses predicted three putative transmembrane domains between about 75-110 aa, 180-220 aa, and 350-370 aa.
- Full length HPL1 fusion constructs appear to target punctate structures in onion epidermal cells
- Based on the co-bombardment with DsRed, the punctate structures appear to be plastids
- There are likely minimal or no targeting mechanisms near the C1 or N1 termini
- Future experiments may investigate deletion constructs of HPL to determine more specific regions responsible for HPL1 localization

Identification of Punctate Structures

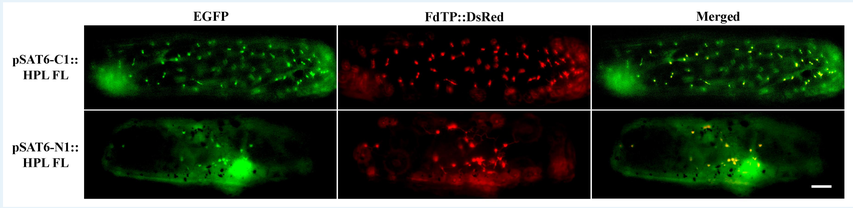

Figure 4: Co-bombardment of onion epidermal cells with EGFP fusion constructs and FdTP::Ds-Red constructs. pSAT6-C1::HPL full length constructs were consistently expressed targeting numerous punctate structures uniform in size, often with "tails". Plastid marker ferredoxin transit peptide and Ds-Red constructs targeted plastids and aligned with the targeting of EGFP. As can be seen in the **Merged** images, overlap of EGFP and DsRed appears yellow. pSAT6-N1::HPL full length constructs also expressed localization to punctate structures, often with lower intensity than that of the pSAT6-C1::HPL constructs. The punctate structures also neatly overlapped with the ferredoxin transit peptide and Ds-Red constructs which identified plastids. Scale bar = 30 μm

References

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