Final Report

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P-type ATPases in Plants – Role of Lipid Flippases in Membrane Biogenesis

The long-range goal of the research is to understand the structure and biological functions of different P-type ATPases (ion pumps) in plant cells, and to use that knowledge to enhance the production of bioenergy from plants, or plant-research inspired technologies. P-type ATPases include ion pumps that specifically transport H^+, Ca^{2+}, Zn^{2+}, Cu^{2+}, K^+, or Na^+, as well as at least one unusual subfamily that appears to function as lipid flippases, flipping specific lipids from one side of a membrane bilayer to the other. As a group, P-type ATPases are thought to consume more than 1/3 of the cellular ATP in typical eukaryotic cells.

Novel and fundamental advances were made in understanding P-type ATPases that transport H^+, Ca^{2+}, Zn^{2+}, Cu^{2+}, K^+, Na^+ and lipids. The previous DOE renewal contains a progress report up to the time of submission (2012). The 2012 renewal application was focused on understanding the biochemical and biological functions of P-type ATPases that flip lipids. Lipid flippases are arguable the least understood members of the P-type ATPase superfamily.

Our DOE sponsored research has pioneered an effort to understand the biological functions of lipid flippases in plants. In collaboration with the Palmgren lab in Denmark, we have now established that this unusual subfamily of P-type ATPases are critical for plants to cope with modest changes in temperature (e.g., down to 15^{o}C, or up to 30^{o}C). In addition, one subclade of these pumps is critical for cell expansion, and loss of function mutants result in severe dwarfism. Although we have a minimal understanding of what the flippases might actually do at the cellular level, they are clearly of fundamental importance to plant growth and response to the environment.

Creating cells that are both source and sinks. One of the innovative ideas proposed in the previous grant was to create a new organelle in plant cells using a strategy involving "zippered membranes". As an update, we have now obtained EMS mutations that appear to stabilize the accumulation of "zippered membranes", and thereby increase the levels of lipids that can be stored in leaf cells. One of our goals is to create new organelles that would provide a stable "lipid sink" that would drive an increase in normal membrane lipid biogenesis. If zippered membranes can be synthesized and stabilized in photosynthetic cells, they create a new lipid sink in the same source cells that capture light energy. In theory, the storage of lipids in photosynthetic cells could provide a significant reduction in the energy costs of transporting photosynthate from a leaf-source to a seed-sink, and thereby maximize the ability to use leaves to directly produce a lipid-based biofuel feedstock.

While our previous results demonstrated that zippered membrane organelles could be generated, and used to provide a modest 20% increase in cellular lipid contents, we realized that higher levels of accumulation would be required if this strategy was to be of significant value to the biofuels industry. In using an EMS mutagenesis strategy we have now identified several independent mutant lines in which zippered membrane organelles appear to accumulate to extremely high levels. These mutant lines are currently being backcrossed to enable continued studies and future genome resequencing to identify the specific mutations. **Our plan is to have preliminary results by the fall of 2016 that would provide a foundation for a new grant proposal aimed at understanding the cellular mechanisms that regulate the synthesis and degradation of membrane lipids.**

Important recent publications supported by DOE funding include:

1. Boursiac Y, Lee SM, Romanowsky S, Blank R, Sladek C, Chung WS, **Harper JF** (2010) Disruption of the vacuolar calcium-ATPases in Arabidopsis results in the activation of a salicylic acid-dependent programmed cell death pathway. ***Plant Physiol.*** 154:1158-71**.**
2. Spalding, E., JF Harper, The ins and outs of cellular Ca transport (2011) ***Current Opinion in Plant Biology***, 14: 715-20.
3. Pedersen CN, Axelsen KB, **Harper JF**, and Palmgren MG. (2012). Evolution of plant p-type ATPases. ***Frontiers in Plant Science***. 3:31
4. Tunc-Ozdemir, M., C Rato, E Brown, S Rogers, A Goyne, S Frietsch, CT Myers, LR Poulsen, R Malhó, **JF Harper** (2013) Cyclic nucleotide gated channels 7 and 8 are essential for male reproductive fertility ***PLOS1*****,** 8 (2):e55277.
5. Tunc-Ozdemir, M, C Tang, MR Ishka, E Brown, N Groves, CT Myers, S McDowell, R Mittler, **JF Harper** (2013) A cyclic nucleotide-gated channel (CNGC16) in pollen is critical for stress tolerance in pollen reproductive development. ***Plant Physiology*** 161: 1010-1020.
6. McDowell, SC, LR Poulsen, RL Lòpez-Marquès, MG. Palmgren, **JF. Harper** (2013) Loss of the *Arabidopsis thaliana* P_4ATPase ALA3 Results in Root, Shoot, and Reproductive Phenotypes that are Strongly Dependent upon Growth Conditions ***PLOS1*****,** 8(5):e62577
7. Limonta M, Romanowsky S, Olivari C, Bonza MC, Luoni L, Rosenberg A, Harper JF, De Michelis MI. (2014) ACA12 is a deregulated isoform of plasma membrane Ca_{2+}-ATPase of Arabidopsis thaliana. ***Plant Mol Biol.*** 84:387-397.
8. McDowell, SC, RL Lòpez-Marquès, T Cohen, E Brown, A Rosenberg, MG. Palmgren, **JF. Harper** (2015) Loss of the *Arabidopsis thaliana* Lipid Flipases ALA6 and 7 alter the lipid composition of pollen and impair pollen tube tip growth. ***Front Plant Sci***. 21:197 (PMID: 25954280)
9. Poulsen, Lisbeth R., Rosa L. López-Marqués, Pai Pedas, Steven McDowell, Elizabeth Brown, Reinhard Kunze, Jeffrey F. Harper, Thomas Günther Pomorski & Michael G. Palmgren (2015) A phospholipid uptake system in the plant Arabidopsis thaliana. ***Nature Communications*** 27;6:7649.
10. Pares R, SC McDowell, RL Lòpez-Marquès, MG. Palmgren, **JF. Harper** (2015) Loss of the *Arabidopsis thaliana* Lipid Flipases ALA4 and 4 reduces cell size and causes dwarfism **(in preparation)**