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Final Technical Report: Chloroplast Dynamics and Photosynthetic Efficiency

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This project investigated the mechanism by which chloroplasts position themselves to maximize solar energy utilization, to enhance gas exchange, to minimize environmental stress, and to promote efficient exchange of metabolites with other compartments within the plant cell. Chloroplasts move within leaf cells to optimize light levels, moving toward levels of light useful for photosynthesis while moving away from excess light. Plastids sometimes extend their reach by sending out projections (stromules) that can connect anchor chloroplasts in position within the cell or provide close contacts with plasma membrane, mitochondria, peroxisomes, endoplasmic reticulum, and the nucleus. The intracellular location of chloroplasts in relation to other organelles with which they share biosynthetic pathways, such as peroxisomes and mitochondria in photorespiration, affects metabolite flow. This work contributed to the knowledge of the mechanisms of organelle movement and anchoring in specific locations in plant cells and how proteins traffic within the cell.

The role of myosin motor proteins in organelle movement and localization.

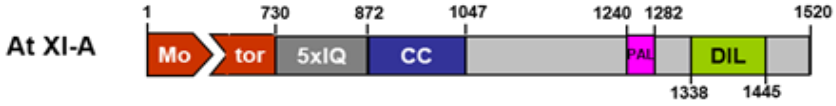
The actin cytoskeleton is known to be important in positioning and movement of plant organelles. Myosin motor proteins operate on actin microfilaments. Plants contain two myosin gene families, myosin XI and myosin VIII; the myosin XI family is similar to the myosin V family, which mobilizes organelles in yeast and animal cells. The Arabidopsis genome contains 13 myosin XI genes and 4 myosin VIII genes.

Various laboratories have previously attempted to identify cargo bound by fluorescence protein (FP)-labeled myosins, with limited success. A few organelles had been successfully labeled by incorporating various regions of myosin tails, but often the localization was cytoplasmic. In other cases, no fluorescent organelles were detected, though a dominant negative effect could be observed, suggesting that the fusion protein was interfering with transport of an organelle but either the protein was in insufficient quantities to be detected, or the fusion resulted in improper folding and lack of fluorescence.

We attempted a new strategy, rather than expressing the whole tail or arbitrary deletion constructs. We identified two domains on 12 of the 13 Arabidopsis myosins that were similar to the vacuole-binding (V) domain characterized in yeast and to the DIL domain characterized in yeast and mouse as required for secretory vesicle or melanosome movement, respectively. Because all of the Arabidopsis regions with homology to the V domain contain the amino acid sequence PAL, we refer to this region as the Arabidopsis PAL domain. We have used the yeast

Myo2p tail structural information to model the 12 myosin XI tail domains containing the homologous PAL and DIL domains.

Fig. 1. Diagram of a typical myosin XI, with motor domain, IQ repeats, CC (coiled-coil) and tail containing the PAL domain and DIL domain. Numbers indicate amino acids.



Yellow fluorescent protein (YFP) fusions were made to each of the 12 PAL and 12 DIL domains and were transiently expressed in *Nicotiana benthamiana* leaves. Remarkably, each such fusion labeled at least one intracellular location, and most labeled multiple locations. Furthermore, dominant negative effects on the labeled organelles were the rule rather than the exception in cells with high expression levels. Dominant negative effects are expected if a non-functional motor can saturate receptors on an organelle, preventing binding of normal motors.

While Golgi, endosomes, and nuclei were labeled by certain PAL fusions and by certain DIL fusions, only PAL fusions labeled mitochondria and only DIL fusions labeled peroxisomes. Identification of these domains will facilitate characterization of the receptors on the respective organelles. Eight YFP::DIL domain fusions labeled peroxisomes; none labeled mitochondria or chloroplasts (Sattarzadeh et al. 2011). Six myosin XI Vacuole domains labeled mitochondria and seven labeled Golgi bodies. Simultaneous transient expression of the PAL sub-domains of myosin XI-H, XI-I, and XI-K resulted in inhibition of movement of mitochondria and Golgi, apparently resulting from a dominant negative effect (Sattarzadeh et al. 2013).

The Arabidopsis myosin XI-F PAL domain and the homologous myosin XI-F PAL domain from *N. benthamiana* labels chloroplasts and stromules in *N. benthamiana* leaves (Sattarzadeh et al. 2009).

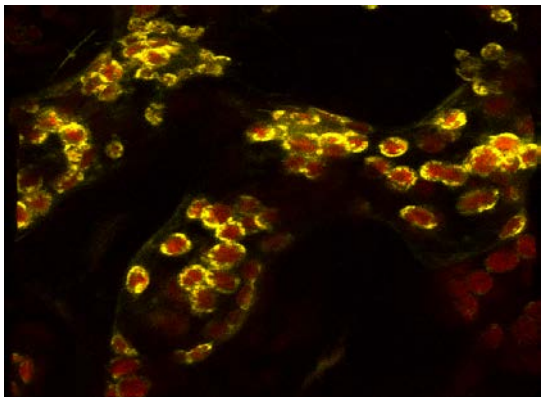


Fig. 2. Yellow signal from YFP::XI-F PAL domain fusion surrounds chloroplasts. Red is chlorophyll autofluorescence. Only the YFP::XI-F PAL domain fusion labels plastids and stromules.

Relevant DOE-supported publications:

Sattarzadeh, A., J. Krahmer, A. D. Germain, M. R. Hanson. 2009. A myosin XI tail domain homologous to the yeast myosin vacuole-binding domain interacts with plastids and stromules in *Nicotiana benthamiana.* Molecular Plant. 2:1351-8

Sattarzadeh, A., E. Schmelzer, and M.R. Hanson. 2011. Analysis of organelle targeting by DIL domains of the Arabidopsis myosin XI family. Frontiers in Plant Genetics and Genomics 2:72.

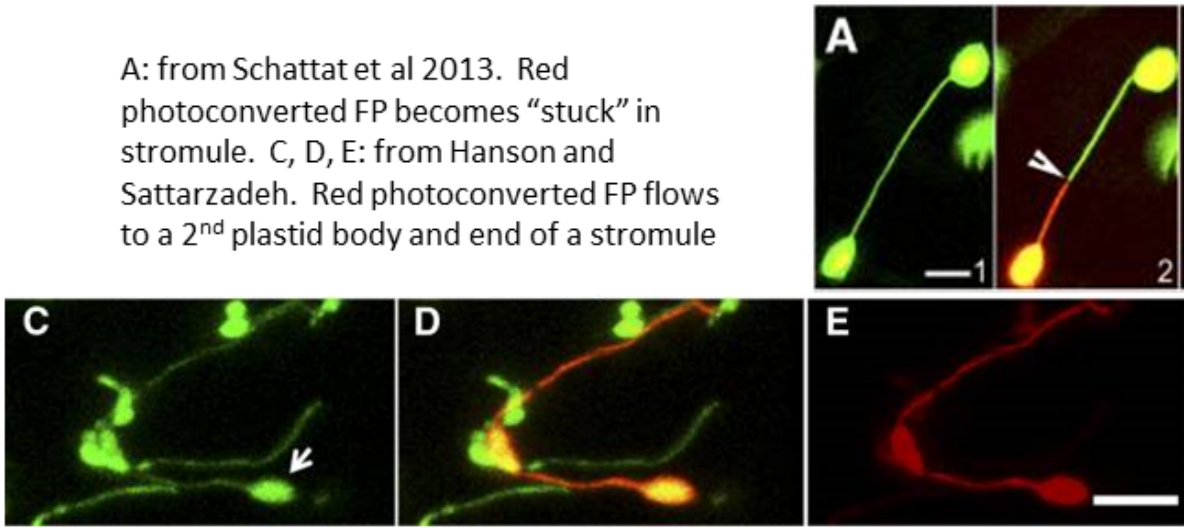
Sattarzadeh, A, E. Schmelzer E, and M.R. Hanson. 2013. Arabidopsis myosin XI sub-domains homologous to the yeast myo2p organelle inheritance sub-domain target subcellular structures in plant cells. Front Plant Sci. 4:407. doi: 10.3389/fpls.2013.00407.

Stromule dynamics and protein flow.

DOE previously funded our research concerning stromule formation and function. In April 2012, a paper appeared from a Canadian group (Schattat et al. 2012, *Plant Cell* 24:1465-77) that claimed our prior DOE-funded work demonstrating flow of proteins from one plastid to another was erroneous. Furthermore, text in this paper stated incorrectly that there was a "dogma" that plastids form an interconnected network, although, in a paper published in 2000, we had demonstrated by photobleaching methods that no such network exists. The authors used a green-to-red photoconvertible GFP while our prior studies had been performed by photobleaching of standard GFP. After writing to the *Plant Cell* editor about our concerns, we were invited to submit a full-length Commentary for peer review that would discuss the omissions in the April 2012 paper, provide new data, and explain why our results differed from the 2012 report.

We obtained the Arabidopsis line containing the photoconvertible GFP from the Canadian group to determine why they failed to observe transfer of protein from one plastid to another nor within a stromule from single plastids. We provided time-lapse movies demonstrating movement of both the photoconvertible GFP and standard GFP between plastids. We believe that the use of high levels of irradiation for photoconversion by the Canadian group resulted in photodamage that prevented them from observing movement of fluorescent protein (**Fig. 3**). Our article appeared in *Plant Cell* (Hanson and Sattarzadeh, 2013).

Fig. 3. Comparison of lack of mEosGFP flow between plastid bodies observed by Schattat et al. using high irradiation for green-to-red photoconversion (A) vs. the rapid flow we observed following pulsed laser light for mEosGFP photoconversion (C, D, E) [35].



Hanson, M.R. and A. Sattarzadeh. 2013 Trafficking of proteins through plastid stromules. Plant Cell. 25:2774-82.

Hanson, M.R. and A. Sattarzadeh. 2011. Stromules: recent insights into a long neglected feature of plastid morphology and function. Plant Physiol. 155:1486-92

Advances in methods for studying chloroplast and protein dynamics.

We have provided two methods papers. One describes how to label plastids with fluorescent proteins to study dynamic morphology and movement (Hanson and Sattarzadeh, 2014). The other describes an unexpected result. While Dr. Sattarzadeh was instructing Reza Saberianfar, a student visiting from Rima Menassa's lab (Agriculture Canada), in photobleaching methods, he accidently discovered that GFP itself could be photoconverted from green to red (**Fig. 4**). Further analysis of multiple cell types as well as *in vitro* studies confirmed this observation. This finding will allow the use of existing transgenic lines expressing standard forms of GFP (plant or animal) to be used in photoconversion experiments to follow protein and organelle dynamics.

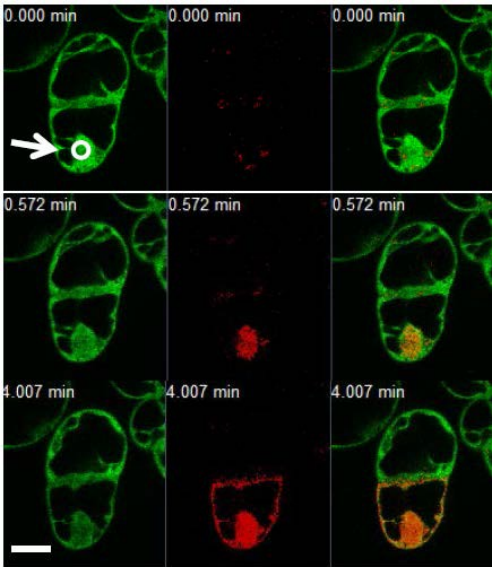


Fig. 4. Photoconversion of EGFP into red state and flow of protein through the cytosol in cultured tobacco cells. Arrow shows where EGFP was irradiated for photoconversion. Bar is 20 µm. (Sattarzadeh et al., 2015)

We also carried out two collaborations that took advantage of confocal microscopy expertise developed during this project. We characterized some transgenic maize lines that were produced in order to specifically label either mesophyll or bundle sheath plastids. Maize plants carrying PepC promoter constructs exhibited YFP expression in mesophyll plastids and the RbcS promoter mediated expression in bundle sheath plastids. The plants carrying PepC and RbcS promoter fusions were found to be useful for identifying plastids in organs such as epidermis, silks, roots and trichomes (Sattarzadeh et al. 2010).

Postdoctoral associate Amir Sattarzadeh also collaborated with Rima Menassa's lab to help characterize protein bodies, which can be induced to form in leaves when foreign proteins are produced at high levels in the endoplasmic reticulum. Proteins could move between protein bodies. This work demonstrated that the ER was the source of three different types of protein bodies and are dependent on myosin for motility (Saberianfar et al. 2016).

Relevant DOE-support publications:

Hanson, M. R. and A. Sattarzadeh. 2014. Fluorescent labeling and confocal microscopic imaging of chloroplasts and non-green plastids. In Pal Maliga (ed.), *Chloroplast Biotechnology: Methods and Protocols*, Methods in Molecular Biology, vol. 1132, Chapter 7. DOI 10.1007/978-1-62703-995-6_7. Springer Science+Business Media New York 2014

Sattarzadeh A., R. Saberianfar, W.R. Zipfel, R. Menassa, M.R. Hanson. 2015. Green to red photoconversion of GFP for protein tracking in vivo. 2015 Sci Rep. 5:11771.

Sattarzadeh, A., J. Fuller, S. Moguel, K. Wostrikoff , S. Sato, S. Covshoff, T. Clemente, M. Hanson, D.B. Stern 2010. Transgenic maize lines with cell-type specific expression of fluorescent proteins in plastids. Plant Biotech. J. 8: 112–125

Saberianfar, R., A. Sattarzadeh, J.J. Joensuu, S.E. Kohalmi, and R. Menassa R. 2016 Protein bodies in leaves exchange contents through the endoplasmic reticulum. Front Plant Sci.7:693.

Review articles concerning photosynthesis.

The P.I. participated in a Banbury Meeting on photosynthetic efficiency. A report was published as the proceedings of this meeting (Ort et al. 2015).

The P.I. also provided a commentary concerning formation of stromules from chloroplasts as a response to oxidative stress (Hanson, 2015).

Relevant DOE-supported publications:

Ort, D.R., S.S. Merchant, J. Alric, A. Barkan, R.E. Blankenship, R. Bock, R. Croce, M.R. Hanson, J.M. Hibberd, S.P. Long, T.A. Moore, J. Moroney, K.K. Niyogi, M.A. Parry, P.P. Peralta-Yahya, R.C. Prince, K.E. Redding, M.H. Spalding, K.J. van Wijk, W.F. Vermaas, S. von Caemmerer, A.P. Weber, T.O. Yeates, J.S. Yuan, X.G. Zhu. 2015. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc Natl Acad Sci U S A. 112:8529-36.

Hanson, M.R. 2015 Reactive oxygen species signal chloroplasts to extend themselves. Proc Natl Acad Sci USA 112:9799-800.