Research interest

Mingjie Chen July 26, 2014

Overview of My Research

Two Topics:

- Metabolic regulation mechanisms in controlling seed storage reserve accumulation and remobilization.
- Plant abiotic stress tolerance.

Five Questions:

- 1. How carbon flux is reprogramed when acetyl-CoA carboxylase (ACCase) is genetically perturbed in developing oil seeds? (Plant Physiology (2009)150: 27-41)
- 2. How seed storage lipid catabolism is integrated with chloroplast biogenesis during seedling establishment? (Plant Cell (2010) 22: 77-90)
- 3. How plant cell membrane lipid desaturation is regulated by low temperature for cold and freezing tolerance? (Plant Cell (2013) 25:1430-1444)
- 4. How plants regulate photoassimilate allocation and partitioning? (Plant Cell (2011) 23: 2991-3006)
- 5. How genotype and environment Interactions shape seed metabolome in corn grain?

Methodology

Multi-dimensional approaches are applied, including:

Forward and reverse genetics

> Biochemistry

Physiology

- Molecular biology
- Cell biology
- Enzymology
- System biology

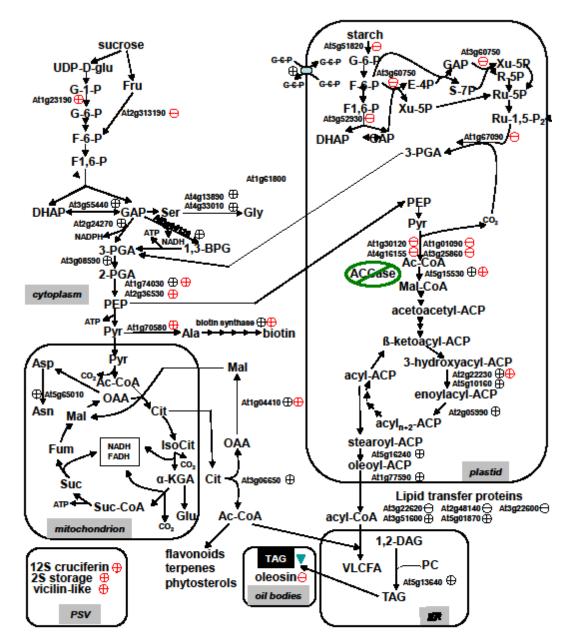
Research projects

- Carbon flux control in oil seeds during seed filling
- Pyrimidine salvage activity for photoassimilate allocation and partitioning
- Storage lipid remobilization during seed germination and seedling establishment
- > Membrane lipid desaturation for plant cold tolerance
- Application of metabolomics to study genetic and environmental interaction

Research interest -----carbon flux regulation during seed filling

Different seeds contain very different composites: rice, corn, and wheat are high in starch; soybean and cotton seeds are rich in protein and oil. The only carbon resource for the seed filling is derived from the photosynthesized sucrose in the leaf; sucrose is transported into developing seeds and is converted into starch, protein, or oil. The metabolic pathways for these seed storage reserve biosynthesis are much known, but the regulation mechanisms for seed composition remains largely elusive. To discover the potential new metabolic regulation mechanisms in developing oil seeds, we applied a targeted genetic perturbation strategy. Acetyl-CoA carboxylase (ACCase) is the rate-limiting step for seed oil biosynthesis during seed filling, and is a protein complex with four subunits. Overexpression of one subunit (BCCP2) under seed-specific napin promoter resulted in reduced ACCase activity, the transgenic seed showed lower oil but higher storage protein contents. The dominant negative nature of this mutant provided unique opportunity to dissect how carbon is channeled into different metabolic pathways for storage oil or protein biosynthesis. I conducted transcriptomic and proteomic studies, then integrated these –omics data. These findings provided novel insights how carbon flux is regulated when oil biosynthesis is perturbed.

The overview of changes in the context of metabolic network in developing seeds



Several interesting observations

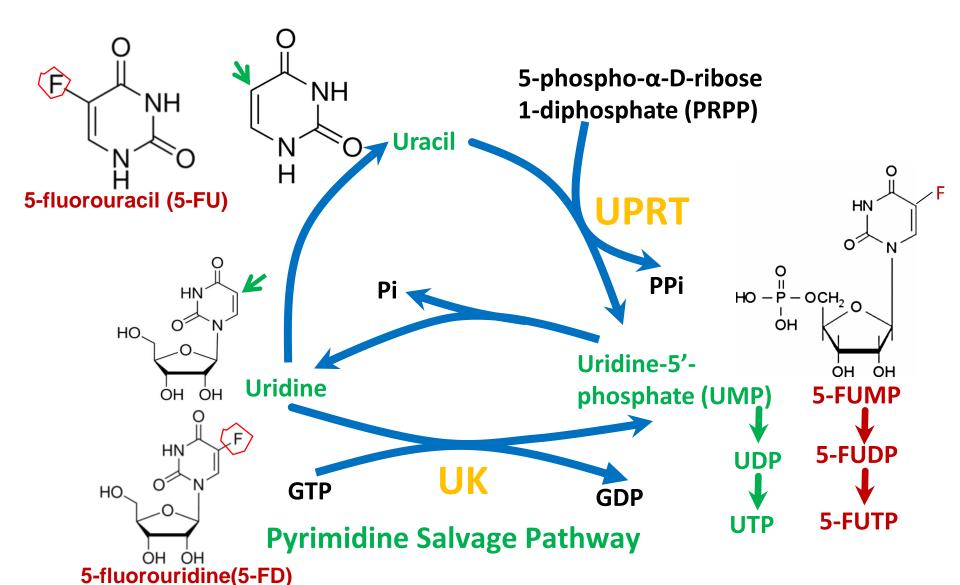
- The plastid glycolysis pathway, which produce the precursor acetyl-CoA for fatty acid biosynthesis, was down-regulated; meanwhile, the cytosolic glycolysis pathway was concurrently upregulated, remodeling of these two pathways may be important to shift the carbon resource from fatty acid biosynthesis to other end products
- TCA cycle was found to play a critical role to control the carbon flux between oil and protein biosynthesis
- The biosynthesis of biotin(a B2 type vitamin), also the cofactor of ACCase, is significantly enhanced.

Research interest

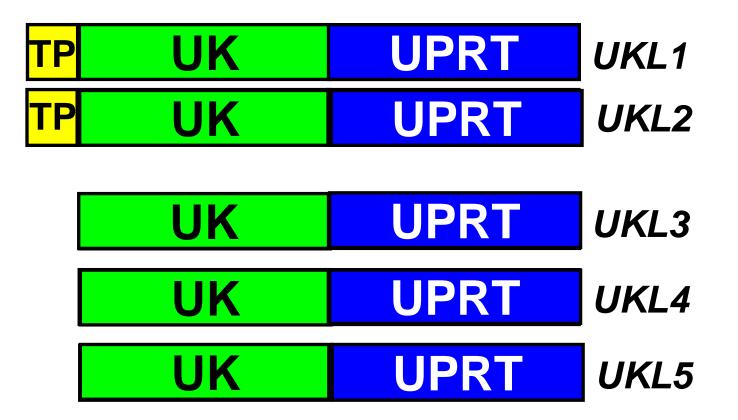
----photoassimilate allocation and partitioning regulation

The autotrophy of higher plants totally depends on their photosynthesis capacity, which harvest solar energy and converts CO_2 and H_2O into carbohydrates. The net product of photosynthesis, D-glyceraldehyde-3-phosphate (GAP), is used for transit starch biosynthesis in the chloroplast, or transported to cytoplasm for sucrose biosynthesis, or moved into mitochondrion for catabolism. The leaf sucrose is exported to sink organs; leaf transit starch is degraded to sustain plant growth during night period. There is a long standing interest to understand the mechanisms for photoassimilate allocation and partitioning, because it's fundamental for plant life and has potential applications for the biomass improvement at both the quantity and quality levels. The biochemical study has demonstrated that glucose is activated by ATP or UTP to form ADP-glucose or UDP-glucose, ADP-glucose is destined for leaf transit starch biosynthesis, and UDP-glucose is used for both sucrose and cellulose biosynthesis, but it remains unclear if the nucleotide metabolism could play a role to regulate photoassimilate allocation and partitioning. All organisms have evolved two different routes to synthesize UTP: the *de novo* biosynthesis use small molecules such as amino acids to form UTP, while salvage pathway recycle the preformed nucleosides or nucleobases to synthesize UTP.

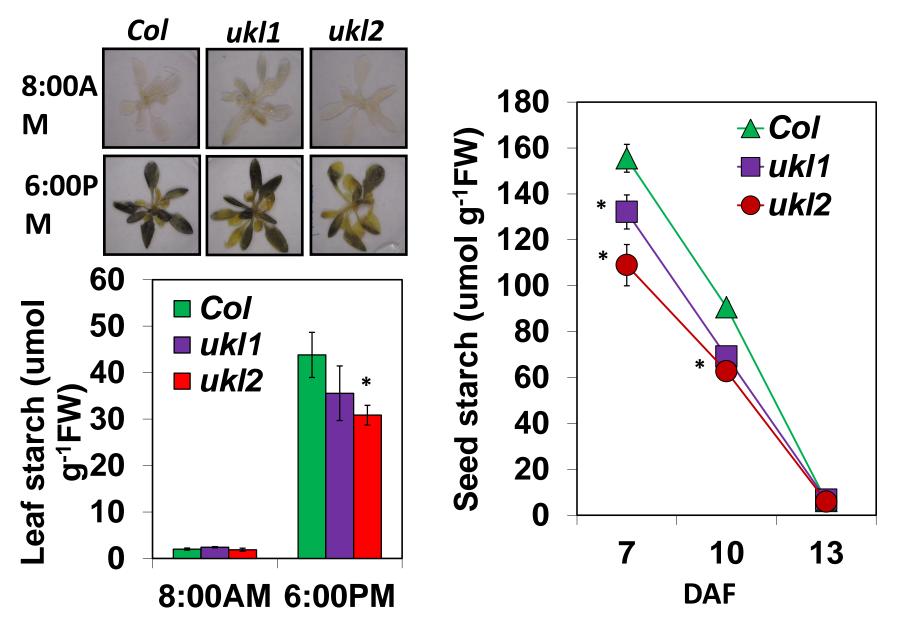
Pyrimidine can be salvaged through Uridine Kinase (UK) and Uracil Phosphoribosyltransferase (UPRT)



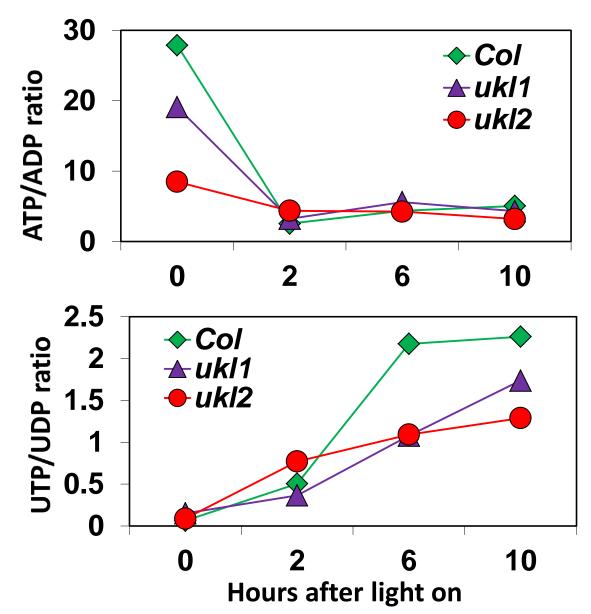
In *Arabidopsis* genome there are five UKLs members, UKL1 and 2 are localizes in plastid



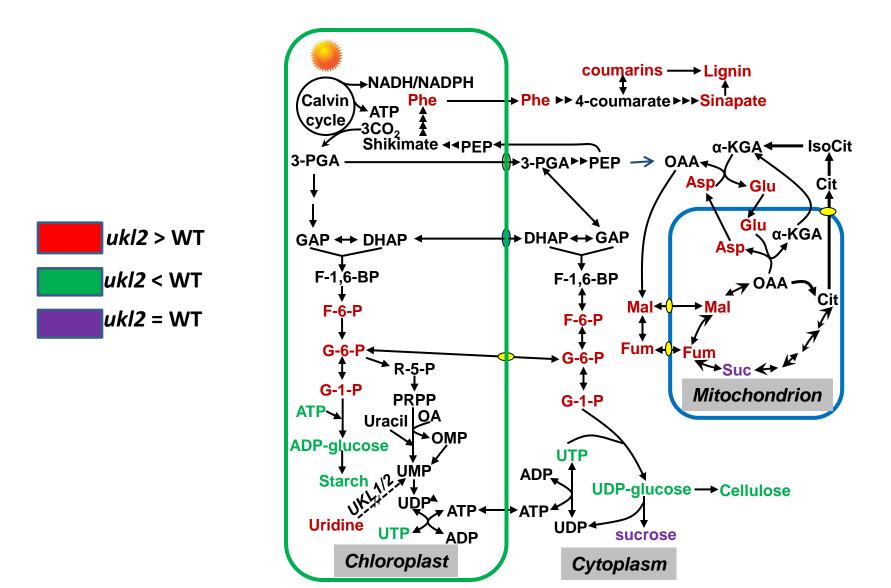
Transit Starch Content in Leaf and Developing Seeds Were Lower in *ukl1* and *ukl2* Mutant Plants



The UTP/UDP Ratio Was Lower in The Mutants Compared With WT



Network Analysis Reveal Metabolic Integration in *Arabidopsis* Leaf



Messages derived from this project

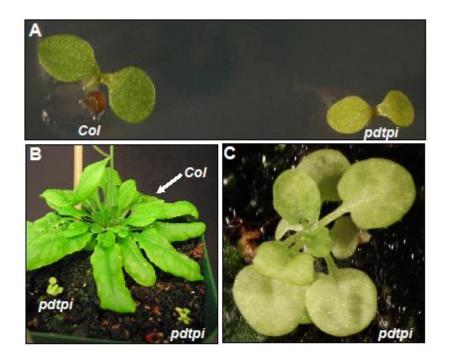
- ✓ UKL1 and UKL2 encode plastid isoform of uridine kinase (UK) but not uracil phosphoribosyltransferase (UPRT); both are functionally redundant.
- ✓ Pyrimidine salvage activity coordinate leaf C- and N- assimilation.
- Metabolic profiling in WT and *ukl2* mutant plants revealed reprograming and integration of multiple metabolic pathways.
- UKL1 and UKL2 are required for fixed-carbon allocation and partitioning in leaves and developing seeds.

Research interest

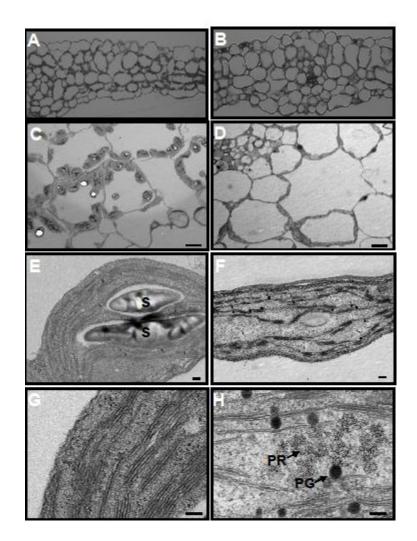
-----Storage lipid remobilization during seed germination and seedling establishment

The packaging of nutrient reserves in seeds has evolved as a strategy to insure the survival of the next generation. These reserves (oil in the form of triacylglycerol, proteins, and carbohydrates) are remobilized to fuel germination and the establishment of a plant's offspring. The seedling establishment and the hypocotyls elongation in darkness are driven by the catabolism of seed storage oil and involves the transition from a heterotrophic to a photoautotrophic seedling, which is manifested by the seedling greening and the biogenesis of a mature chloroplast. Establishment and growth of a seedling not only require the breakdown of energy-rich compounds, but also the synthesis of an array of metabolites for growth. Thus, during this transit early seedling stage, the reserve mobilization should be closely coordinated with its developmental program. Unfortunately, it still largely remains unclear how the seed carry out this metabolic integration. I made contribution to this knowledge gap by identifying a plastid isoform of triose phosphate isomerase (pdTPI), and demonstrated that pdTPI plays an essential role to integrate oil reserve mobilization with seedling establishment and chloroplast biogenesis. By understanding the mechanisms to regulate chloroplast biogenesis, one would be capable of improving the seedling vigor, and this should lead to a better growth performance of crop species.

Partial loss of pdTPI activity compromise seedling establishment and growth



Chloroplast morphology and ultrastructure altered in mutant (B,D,F,H) comparing with WT(A,C,E,G)

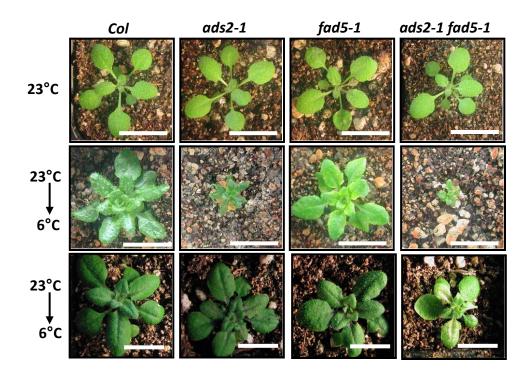


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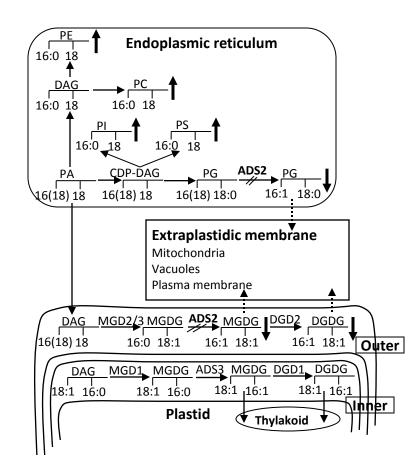
-----Membrane lipid desaturation for plant cold tolerance

Low temperature is one of the major environmental factors restricting plant geographic distribution, growing season and productivity. One strategy that plant used to combat with low temperature is to increase the membrane fluidity through the enhancement of the membrane lipid unsaturation. But the enzymes responsible for this membrane lipid desaturation remained largely unclear for a long period. By a combination of genetic, cellular, biochemical, and lipidomic approaches, I identified that a cold-inducible acyl-lipid desaturase 2 (ADS2) is playing crucial role for cold and freezing tolerance in *Arabidopsis*. When *ADS2* gene was knocked out, the mutant plants grew much slower than WT and were also sterile under low temperature . At the biochemical level I identified that PG and MGDG are the substrates for ADS2 desaturation. The defects in membrane lipid desaturation in *ads2* mutant plants were associated with their compromised growth under cold stress conditions.

Mutation in ADS2 resulted in reduced cold tolerance



Schematic Representation of the Roles Played by ADS2 in MGDG and PG Biosynthesis in *Arabidopsis* Leaves



Future directions

Expand our knowledge of metabolic regulation from model system to other important horticulture or crop species

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