Metabolomics, a Useful Technique for Functional Genomics

Metabolomics Workshop, University of Missouri

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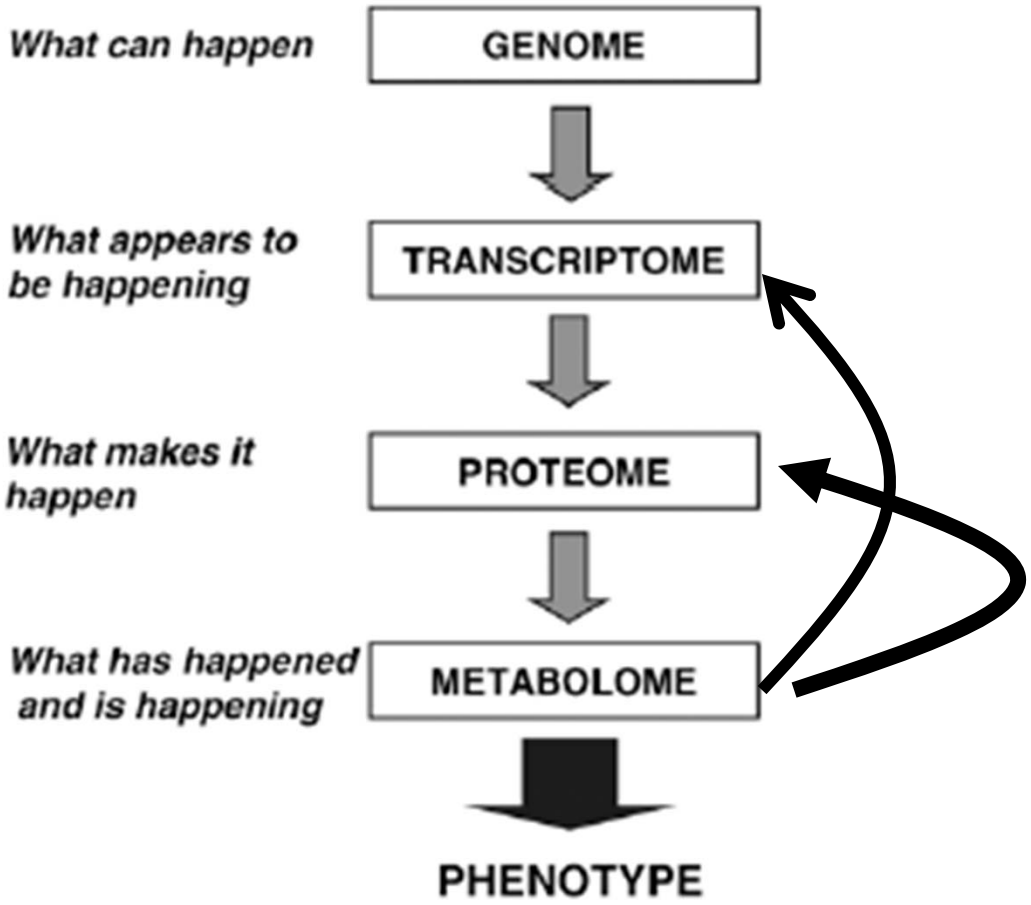
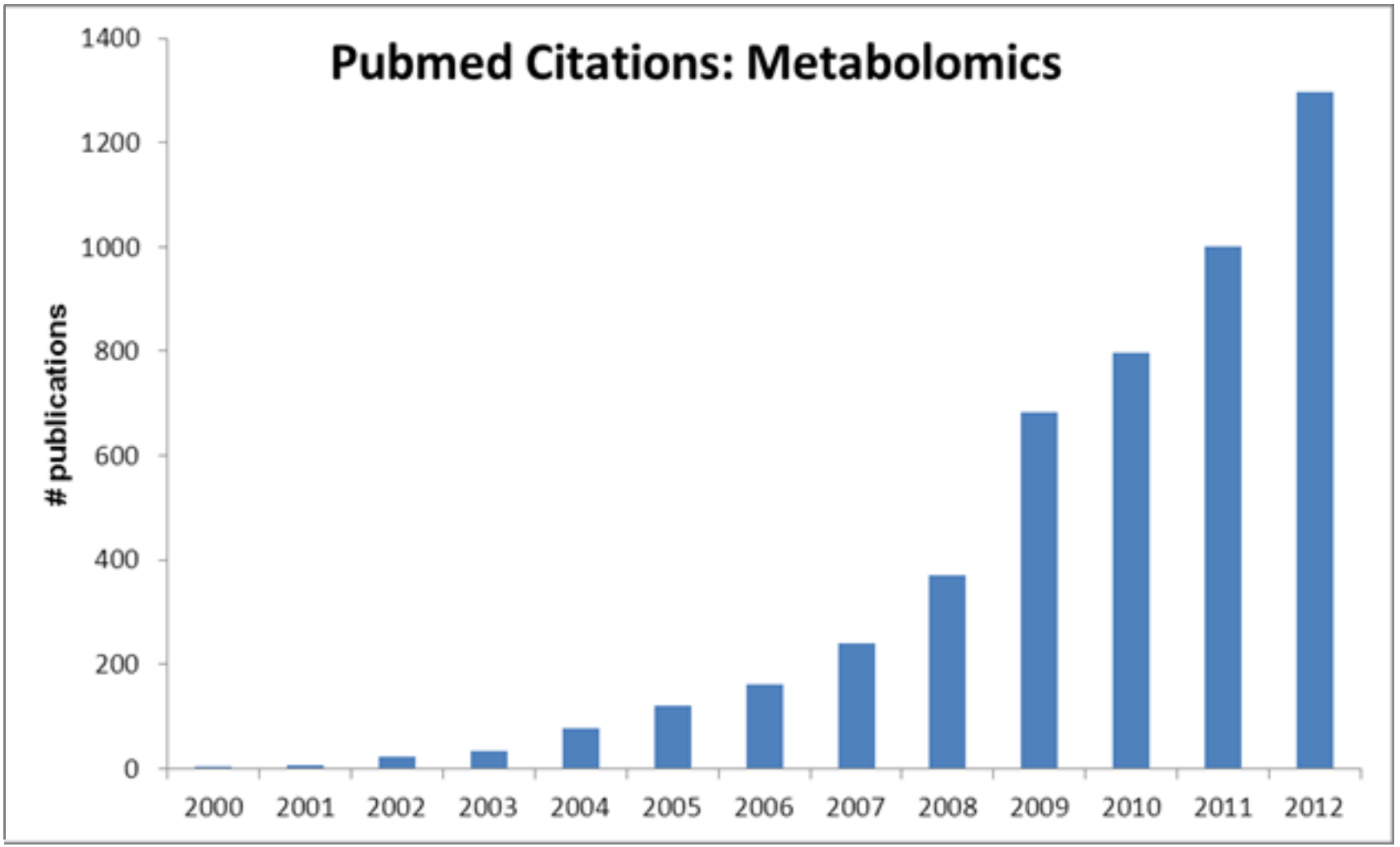


- Metabolome is the overall results of the regulation network, thus is the most predictive of phenotype.

- Many metabolites serve as signaling molecules to regulate transcriptome and proteome. Thus, metabolomics has potential to offer a deeper insight into the regulation network.

- Metabolomics also can be helpful for functional prediction of unknown genes.

Metabolomics are widely applied in Biomedical research



A case study—
putative uridine kinase /uracil phosphoribosyltransferase (UK/UPRT)

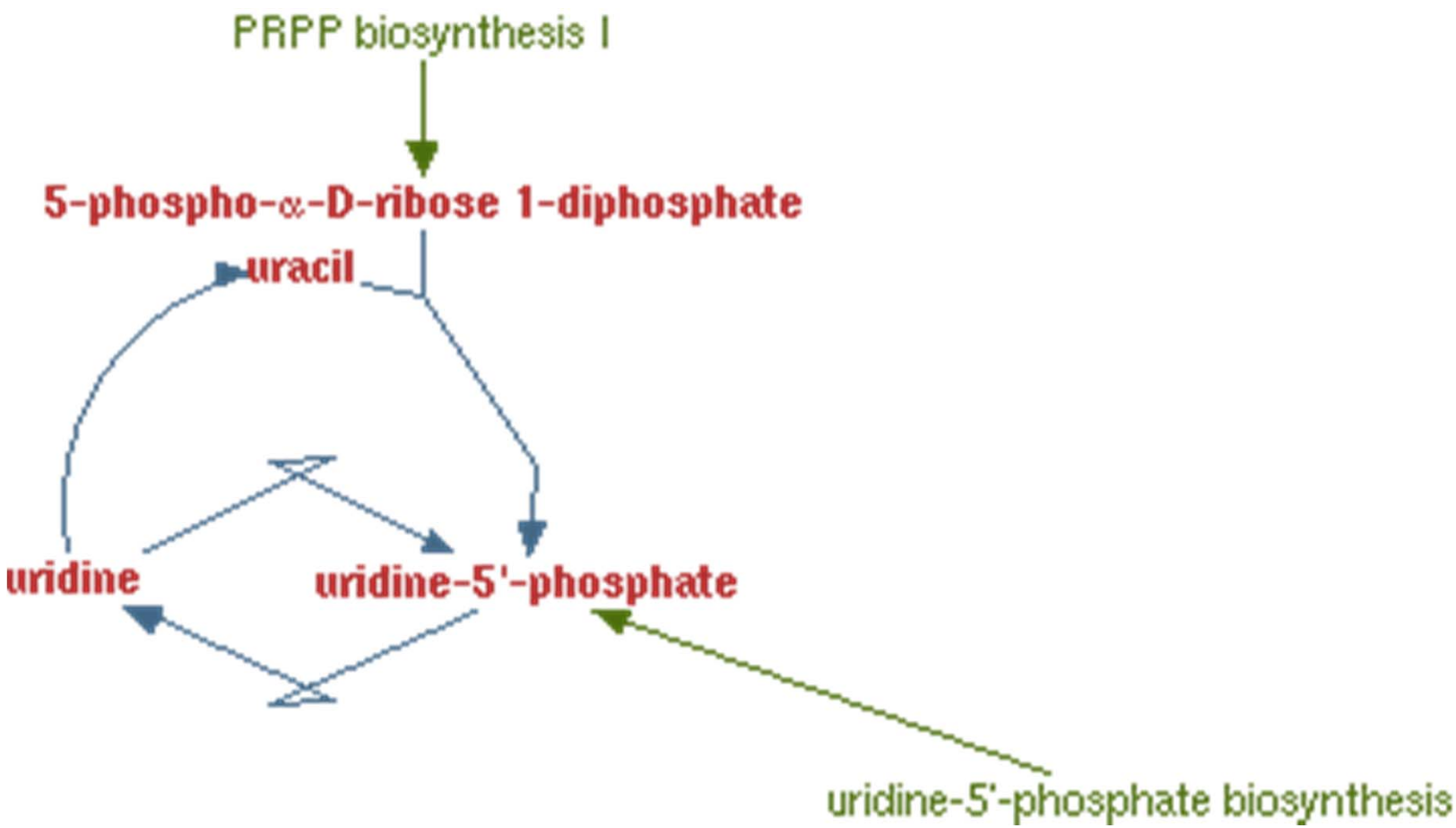
Background & Rationale

- **Uridine triphosphate nucleotide (UTP) is one of the essential nucleotides for cellular growth**
- **Uridine kinases (UK) and uracil phosphoribosyltransferase (UPRT) are involved in salvage pathways for UTP synthesis**
- **Cellular and biochemical regulation of UTP salvage pathway is unclear in plants**

UK and UPRT are involved in pyrimidine salvage pathway

uridine + GTP <=> uridine-5'-phosphate + GDP + H+

uracil + 5-phospho-α-D-ribose 1-diphosphate ⟶ uridine-5'-phosphate + diphosphate



Five genes encoding putative UK/UPRT in the reference plant *Arabidopsis thaliana*

```
UKL5            -----------MEKlSn----GvR-dhcLISdy-----------------vSpSaPApl
UKL1           MPEdSsSldyAMEKASgpHFSGLR-fDGLLSSsPPnSsvvsSlrsavSSSspSsSDPeap
UKL2           MPEdStaidyvMEKASgpHFSGLR-lDGLLSSpskSSvsspShfrlsNSSfsAtdDPAap
UKL3            --maSKSdvniiEtSSkvHFSGfhqmDGLaSNrP----------------eqmAEeeeh
UKL4            --mgSKSvvdmiEaASraHFSGLh-vnGhMnglePSalke----------tTsAsediq

UKL5           kQPFVIGVAGGTASGKTTVCnMImsQLHDQRVVLVNQDSFYHsLTkEkLnKVHEYNFDHP
UKL1           kQPFiIGVsGGTASGKTTVCDMIIQQLHDhRVVLVNQDSFYrGLTsEELqRVqEYNFDHP
UKL2           hQPFVIGVtGGTASGKTTVCDMIIQQLHDhRIVLVNQDSFYrGLTsEELehVqEYNFDHP
UKL3           gQPFVIGVAGGaASGKTTVCDMImQQLHDQRaVvVNQDSFYHnvnEvELvRVHdYNFDHP
UKL4           rQPFVIGVAGGaASGKTTVCDMIIQQLHDQRVVLiNlDSFYHnLTEEELaRVHEYNFDHP
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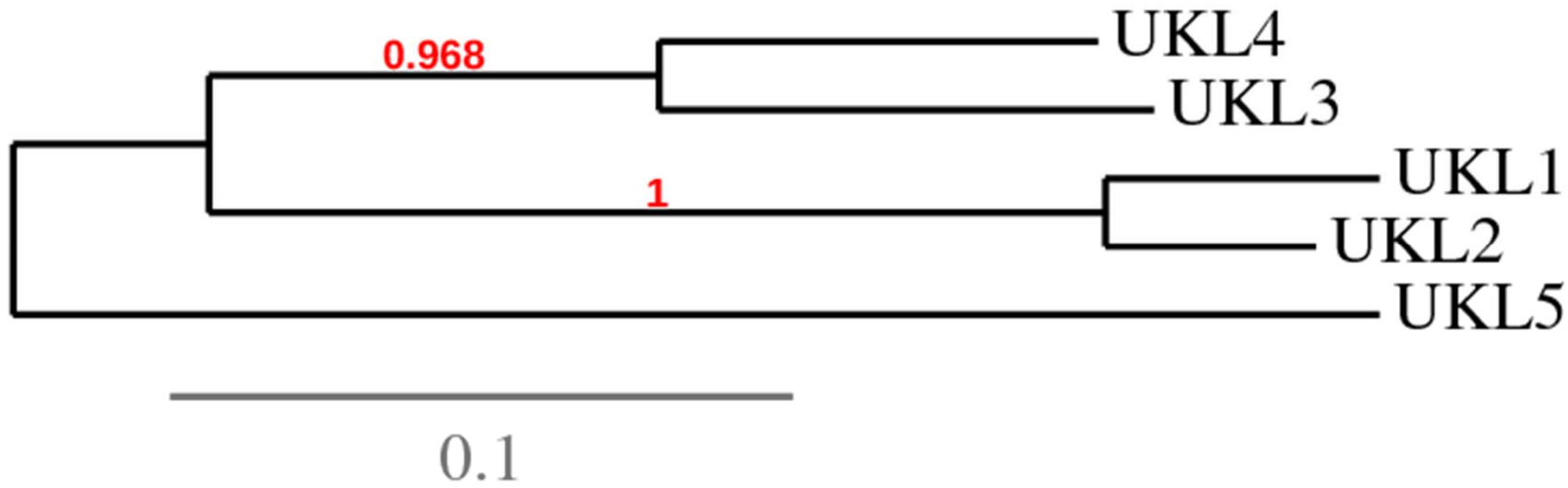
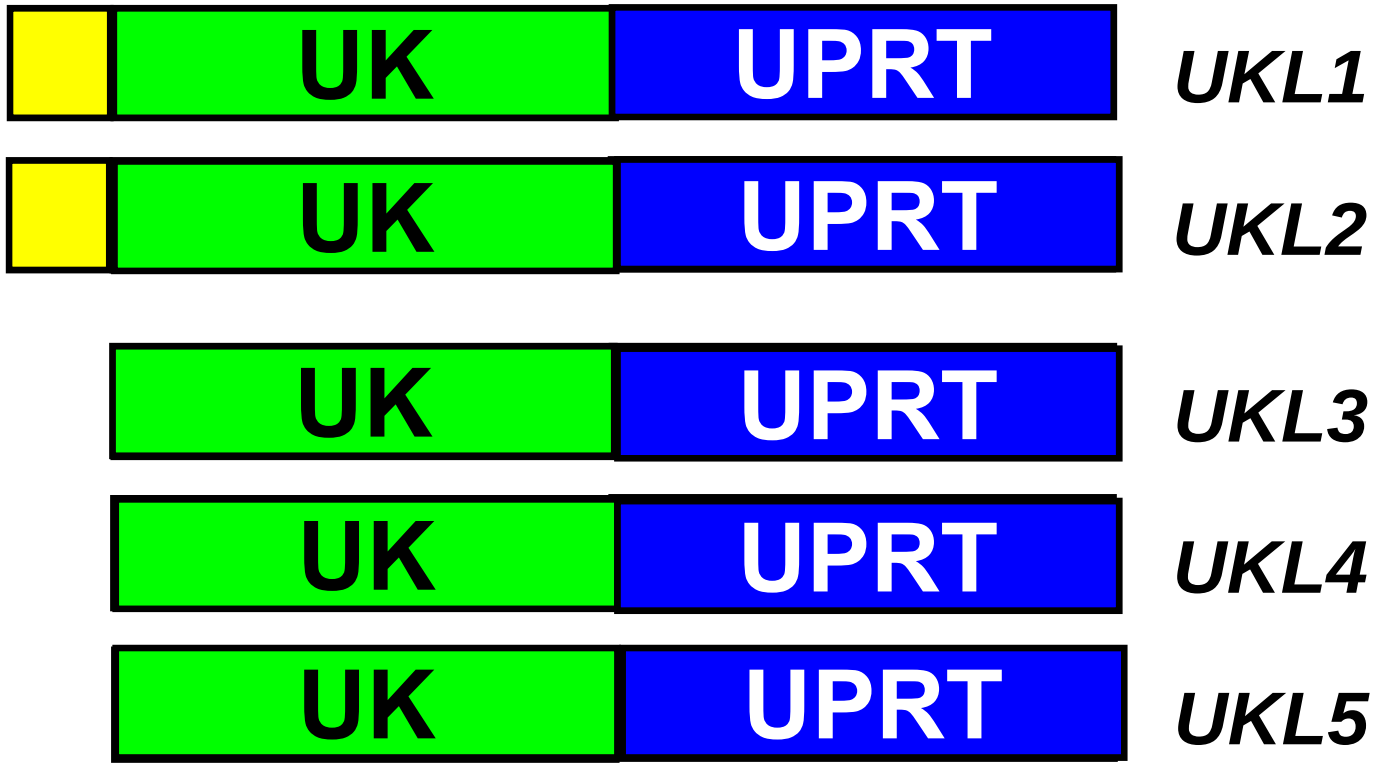
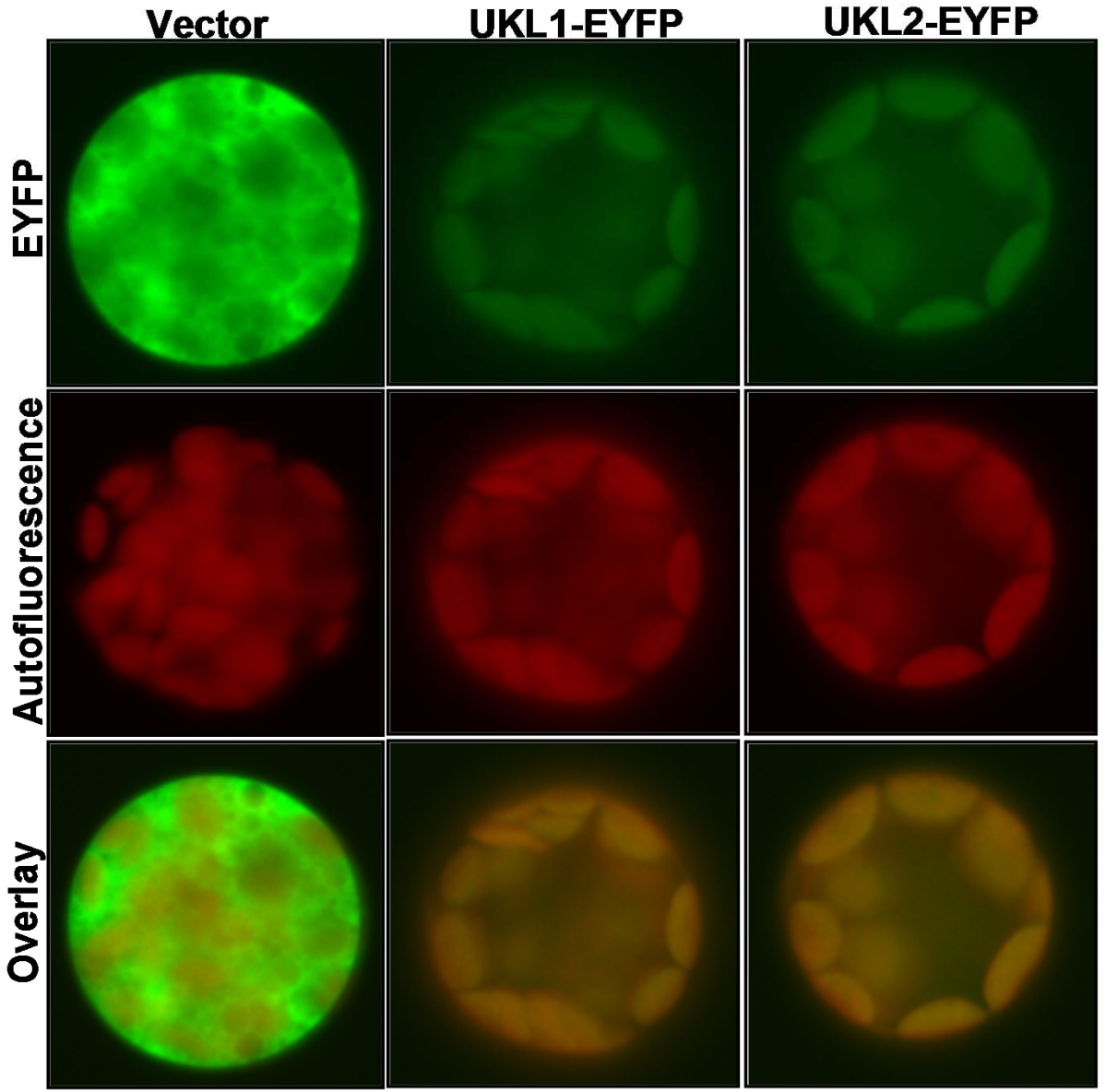


UKL family has UK and UPRT domain. Two genes have a putative targeting peptide.

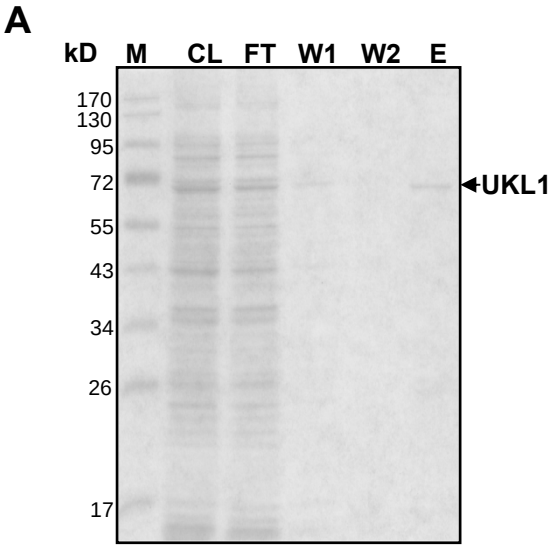


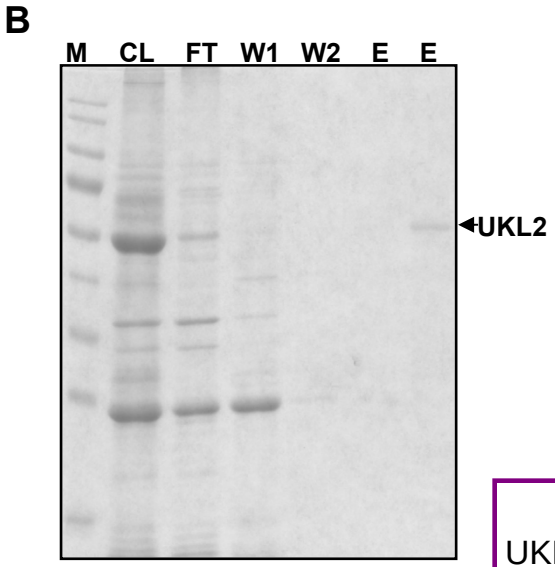
UKL1 and UKL2 localize to plastid



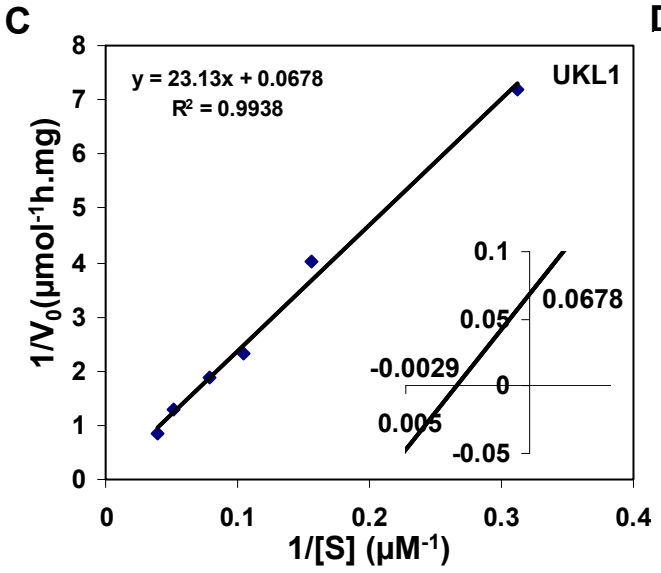
Chen and Thelen (2011). Plant Cell 23:2991-3006.

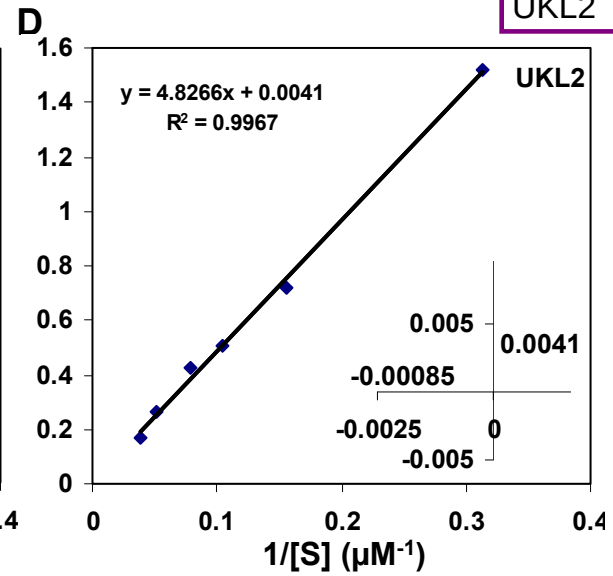
UKL1 and UKL2 only have uridine kinase (UK) activity *in vitro* assay





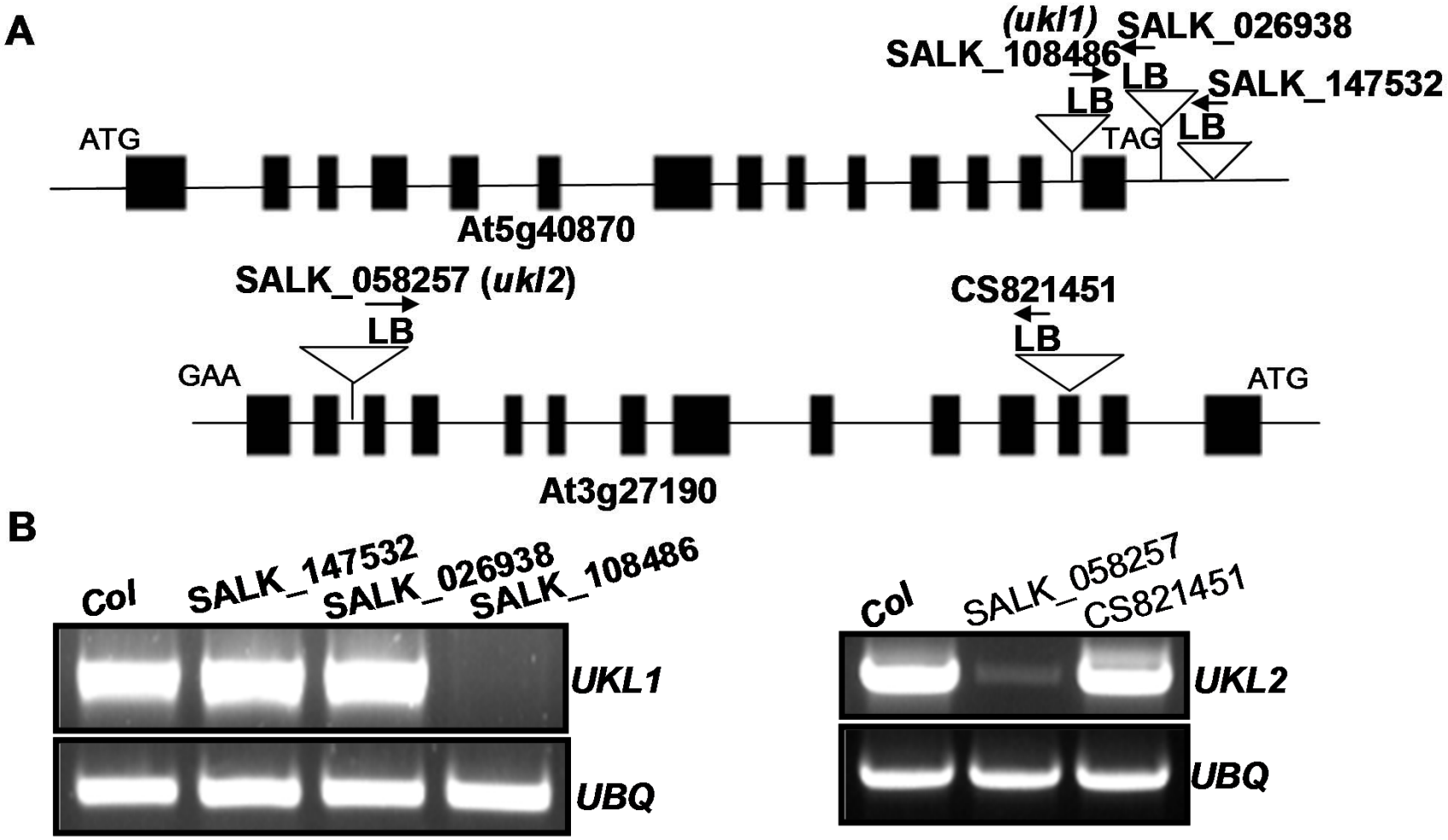
	Km(mM)	Vmax(µmol.h^{-1}.mg^{-1})
UKL1	0.34	14.8
UKL2	1.18	243.9





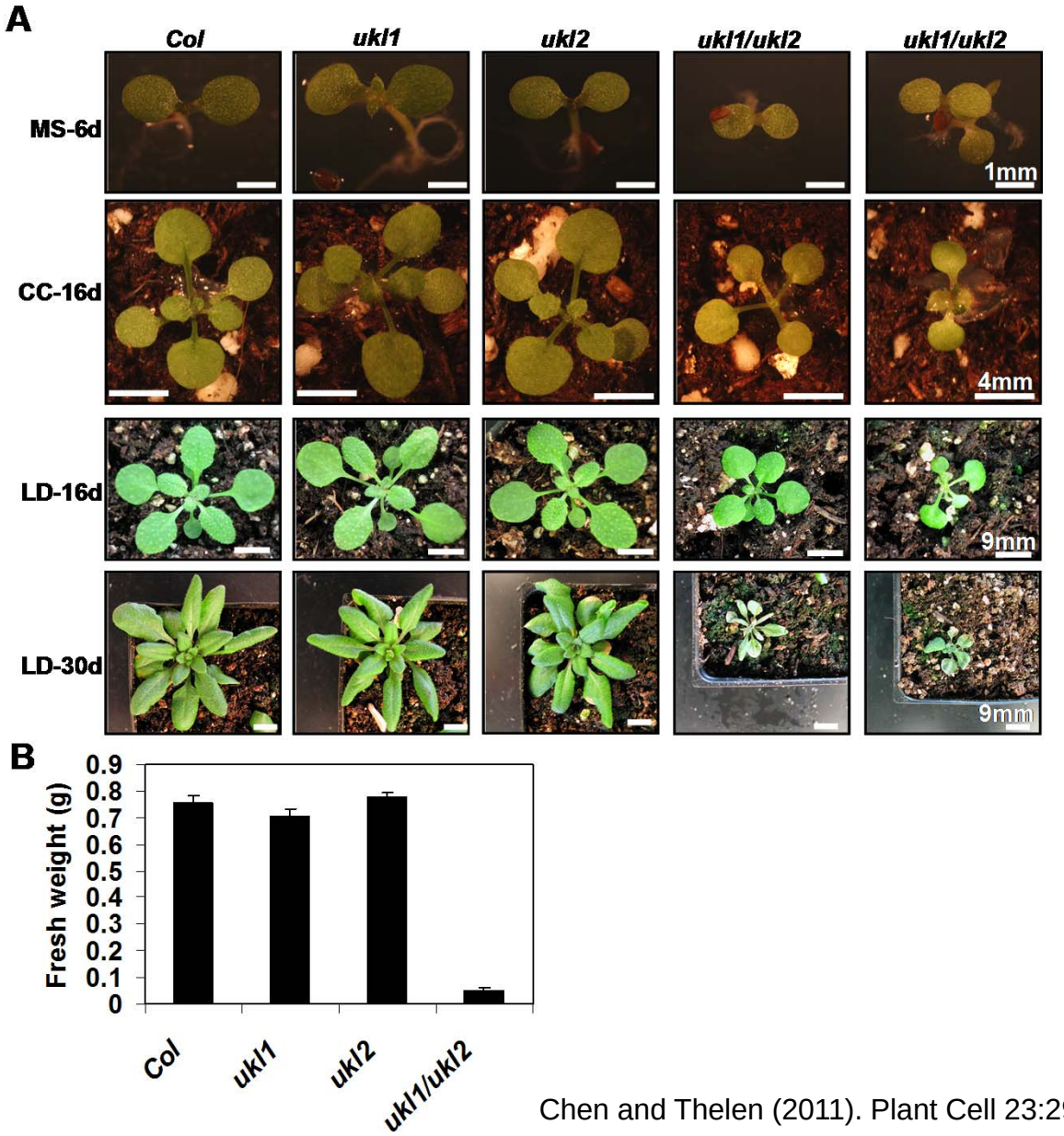
Chen and Thelen (2011). Plant Cell 23:2991-3006.

T-DNA knockout plants on *UKL1* and *UKL2* genes were identified



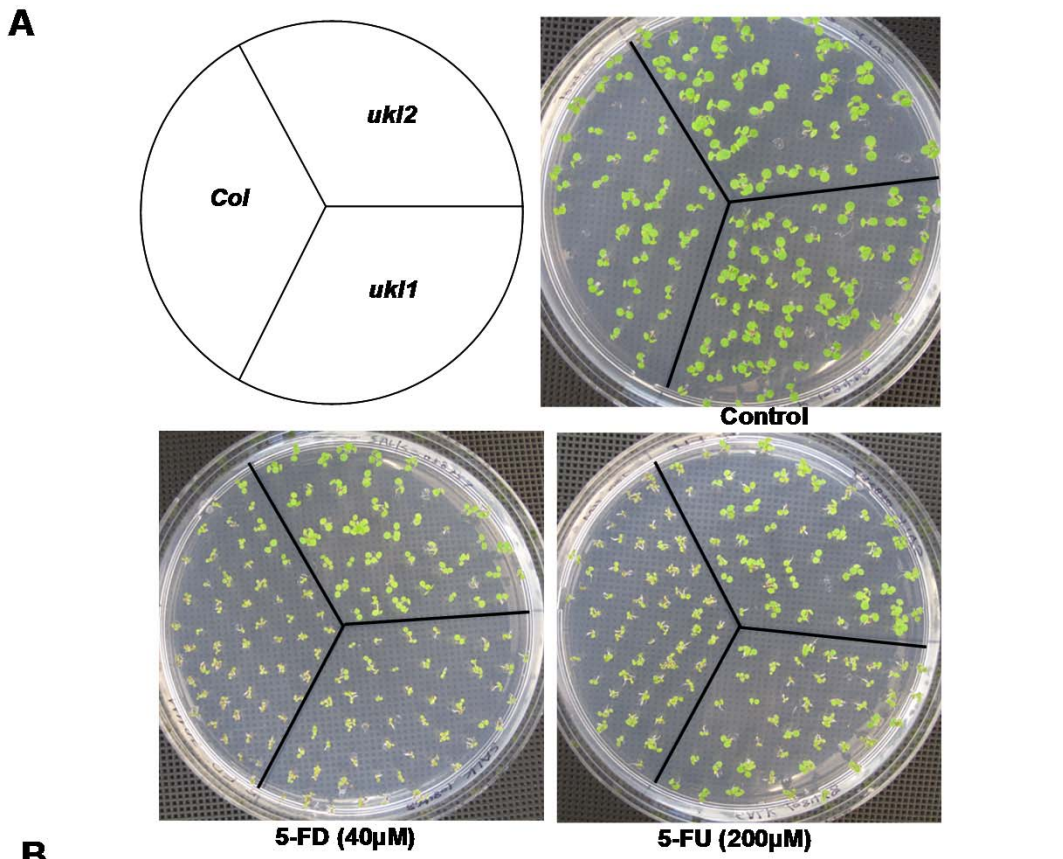
Chen and Thelen (2011). Plant Cell 23:2991-3006.

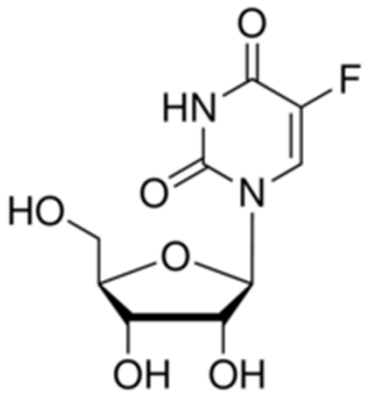
ukl1 and *ukl2* single mutant plants did not show growth defects



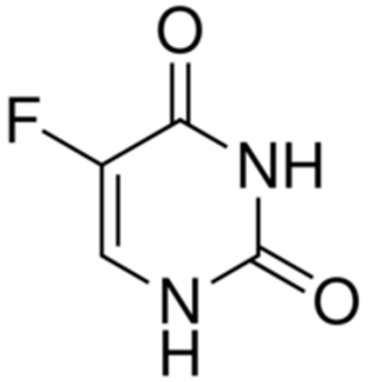
Chen and Thelen (2011). Plant Cell 23:2991-3006.

ukl1 and *ukl2* mutant plants show reduced sensitivity to 5-FD and 5-FU





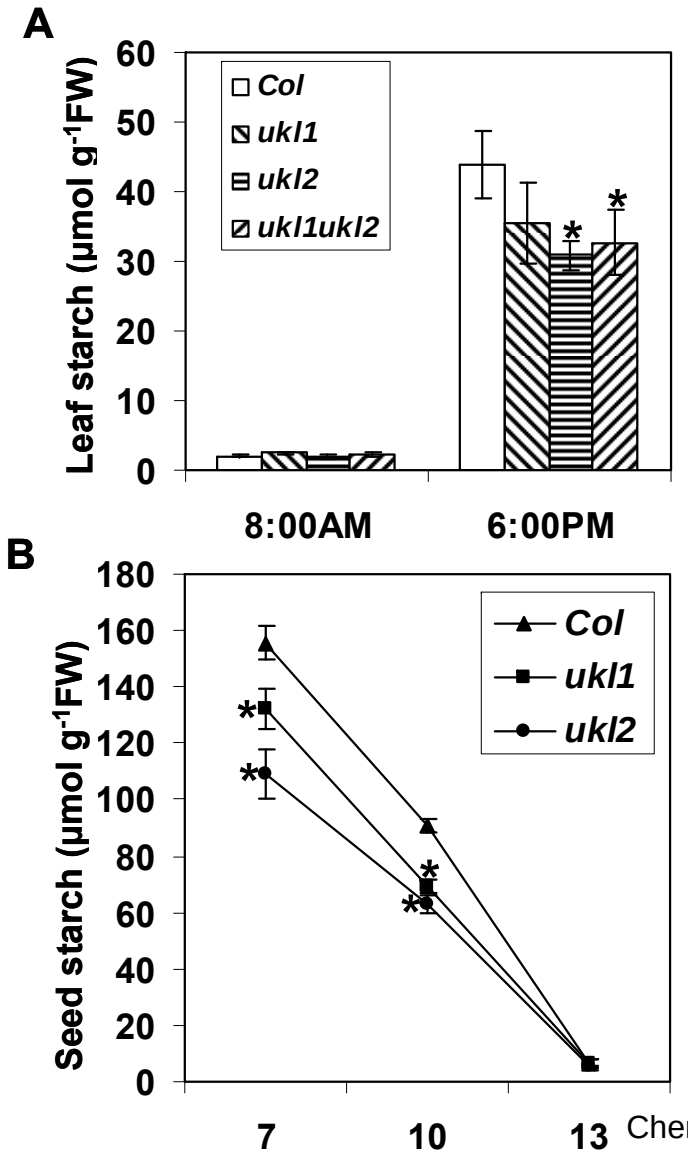
5-Fluorouridine



5-Fluorouracil

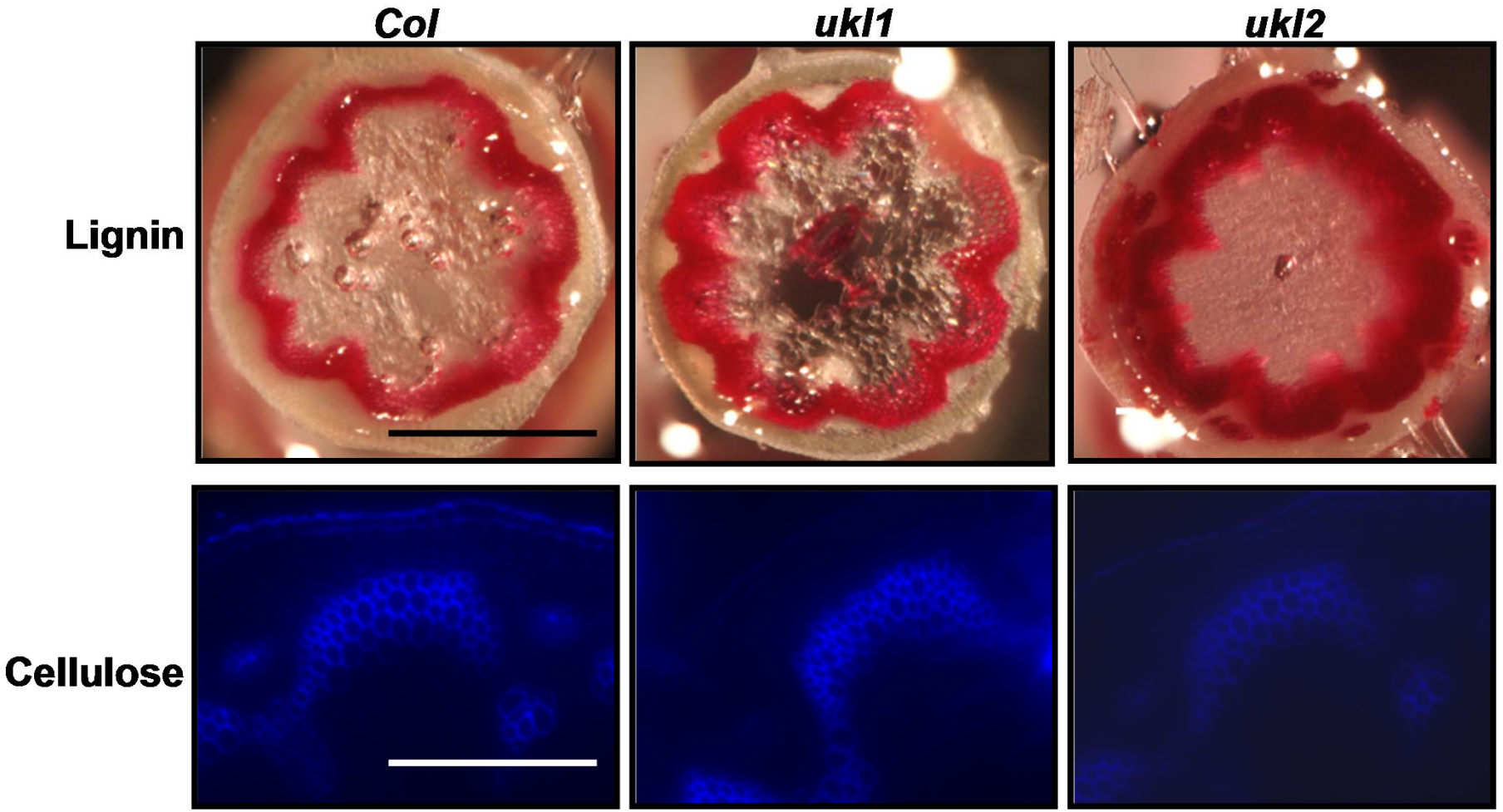
Chen and Thelen (2011). Plant Cell 23:2991-3006.

Transit starch accumulation in leaf and developing seeds were reduced in *ukl1* and *ukl2* mutant plants



Chen and Thelen (2011). Plant Cell 23:2991-3006.

Stem has higher lignin but lower cellulose content in *ukl2* mutant plants



Chen and Thelen (2011). Plant Cell 23:2991-3006.

The seed composition in *ukl1* and *ukl2* mutant plants is altered

Table II. *ukl1* and *ukl2* Seed Trait Analysis

	Col	***ukl1***	***ukl2***
Total silique/plant	193.3 ± 23.0	191.2 ± 10.0	184.5 ± 11.9
Seed weight (mg)/plant	112.2 ± 10.5	104.4 ± 5.1	118.0 ± 8.0
Seed weight (mg)/1000 seed	20.4 ± 0.8	21.8 ± 1.0*	22.3 ± 0.6*
Lipid (%)	38.0 ± 1.1	32.8 ± 0.5*	33.6 ± 1.1*
Protein (%)	38.8 ± 0.3	40.9 ± 0.7*	41.5 ± 0.7*
Starch (%)	0.25 ± 0.01	0.29 ± 0.01*	0.27 ± 0.03
Sucrose (%)	1.79 ± 0.22	2.68 ± 0.28*	2.18 ± 0.12
Glucose (%)	0.32 ± 0.06	0.32 ± 0.07	0.32 ± 0.06
Fructose (%)	0.74 ± 0.05	0.78 ± 0.08	0.75 ± 0.04

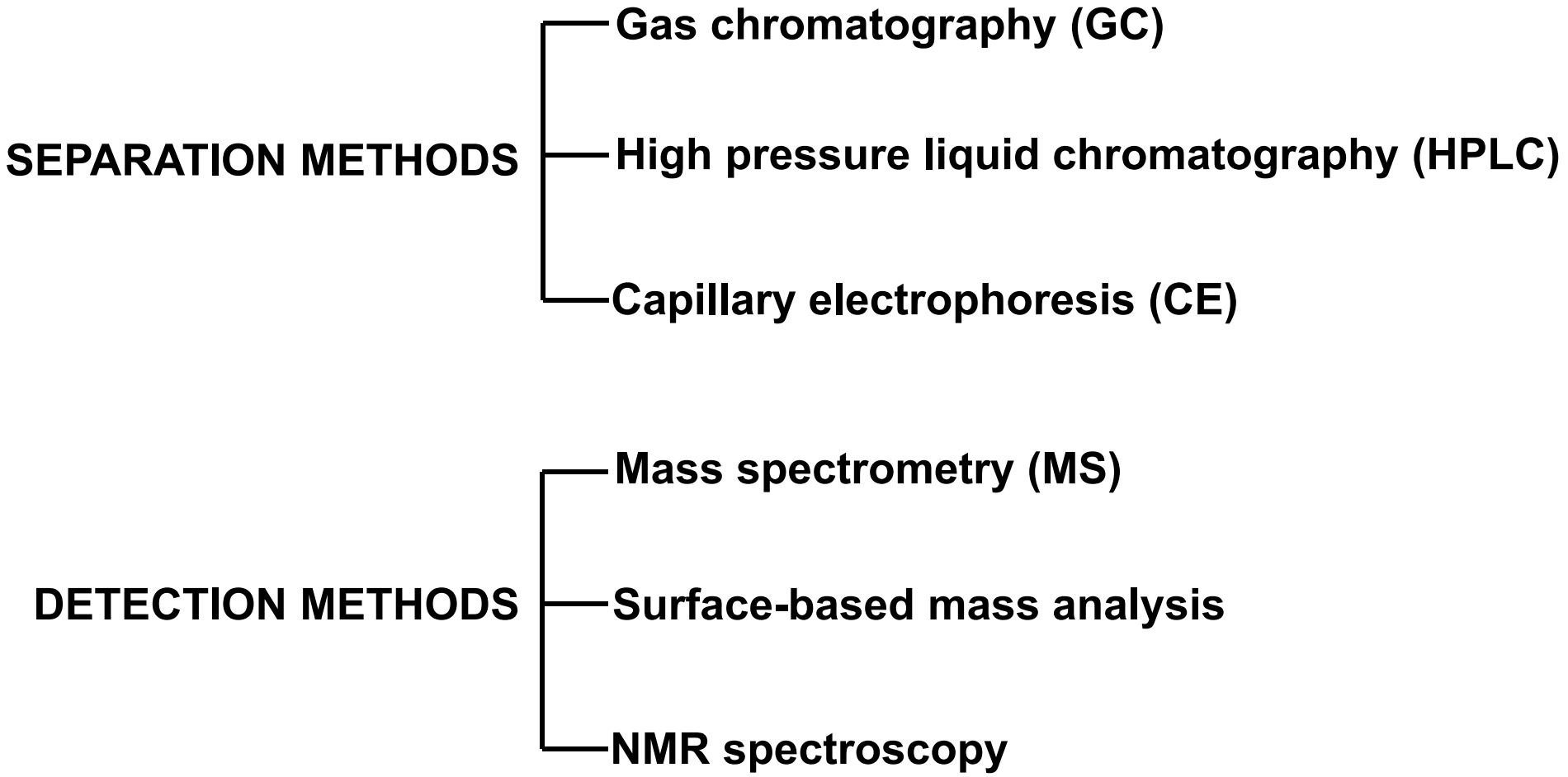
Data is expressed as means ± standard error (n =5). *p* value (total silique per plants) is 0.47(*ukl1*) and 0.39 (*ukl2*); *p* value (total seed weight per plant) is 0.3(*ukl1*)and 0.36 (*ukl2*); *p* value (seed weight per 1000 seeds) is 0.043(*ukl1*)and 0.047 (*ukl2*); the *p* value (lipid) is 0.003 (*ukl1*) and 0.02 (*ukl2*); *p* value (protein) is 0.0102 (*ukl1*)and 0.0028 (*ukl2*); *p* value (starch) is 0.013 (*ukl1*) and 0.22 (*ukl2*); the *p* value (sucrose) is 0.037(*ukl1*) and 0.14 (*ukl2*); the *p* value (glucose) is 0.41(*ukl1*) and 0.52 (*ukl2*); the *p* value (fructose) is 0.07(*ukl1*) and 0.08 (*ukl2*).

Chen and Thelen (2011). Plant Cell 23:2991-3006.

Why did we do metabolomics?

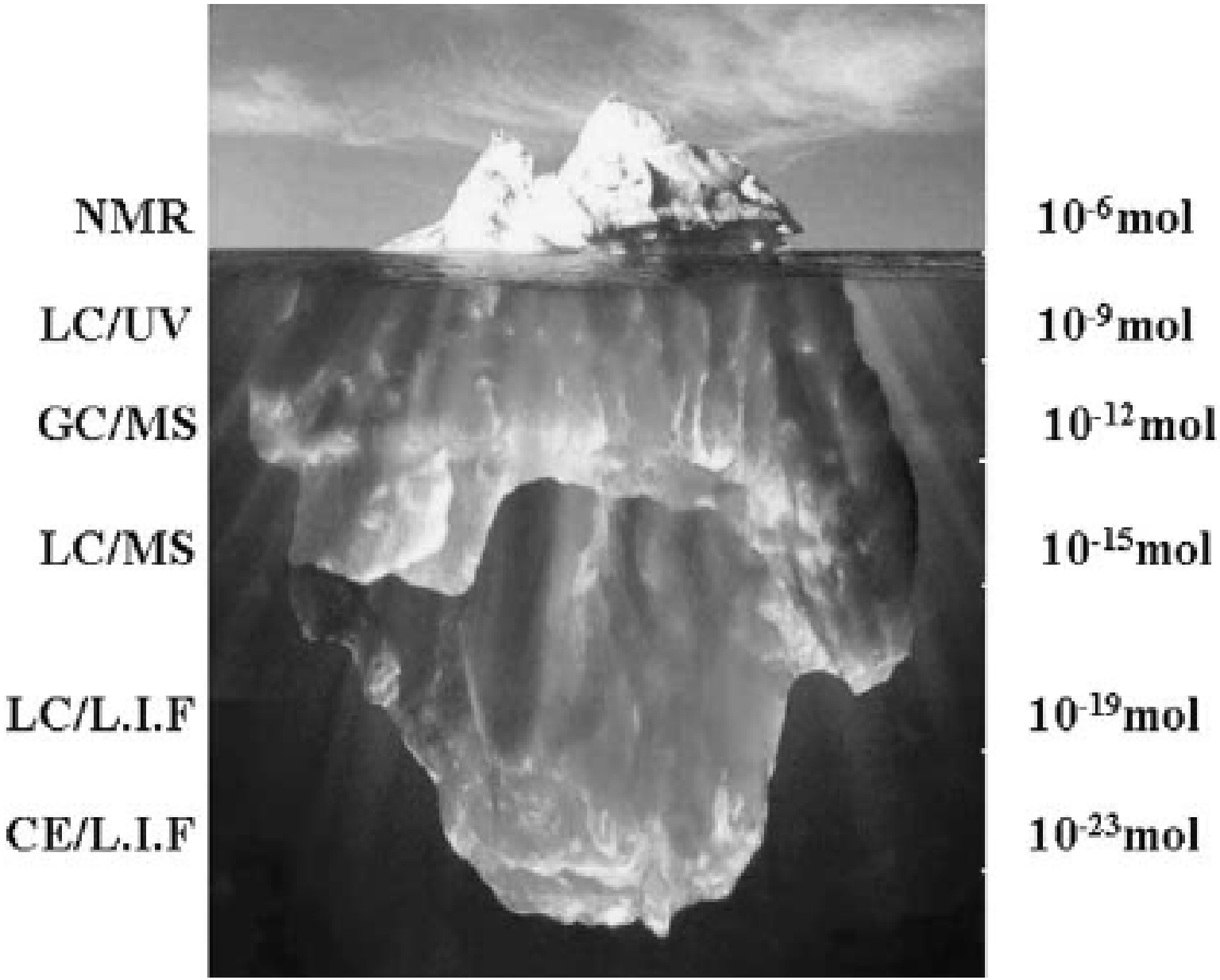
- No obvious visual phenotype for single mutants
- However, what cause the biochemical phenotypes? are there metabolite differences?
- Multiple approaches to interrogate the metabolome… which to use?

Analytical Technology Platforms

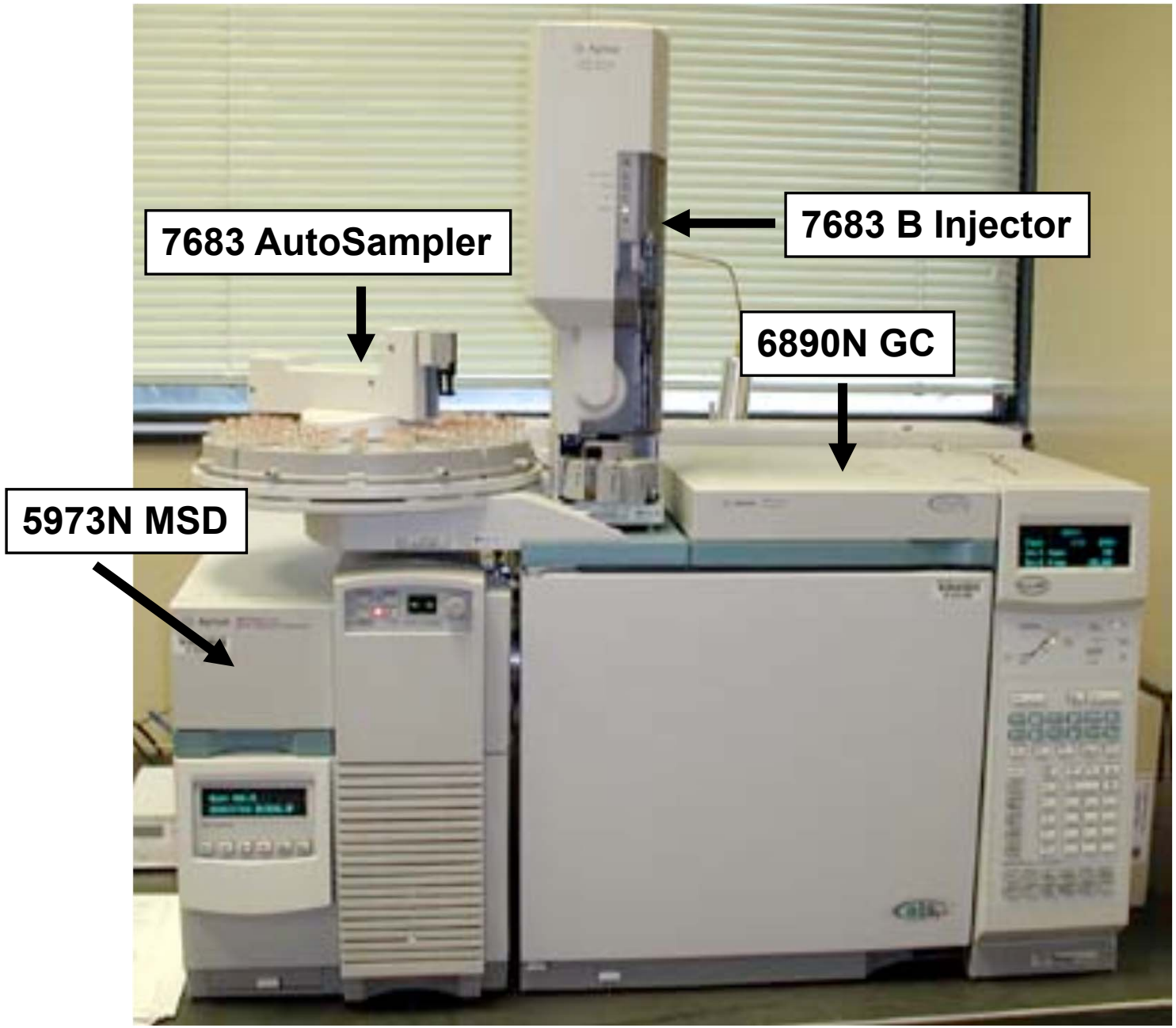


Note: Each platform has properties both unique and complementary advantages.

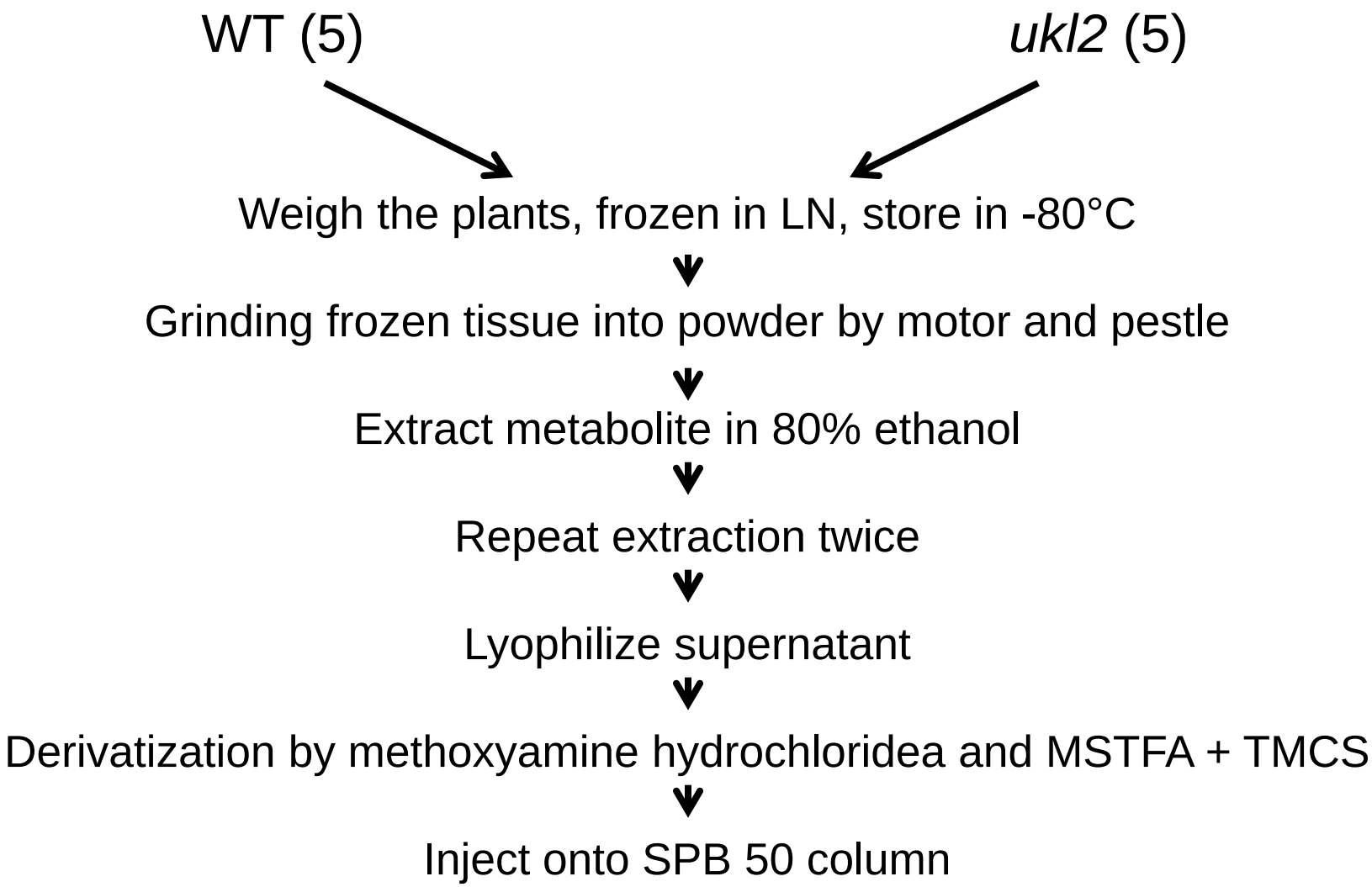
Also…metabolomic approaches differ widely in sensitivity



Metabolomic analysis by GC-MS

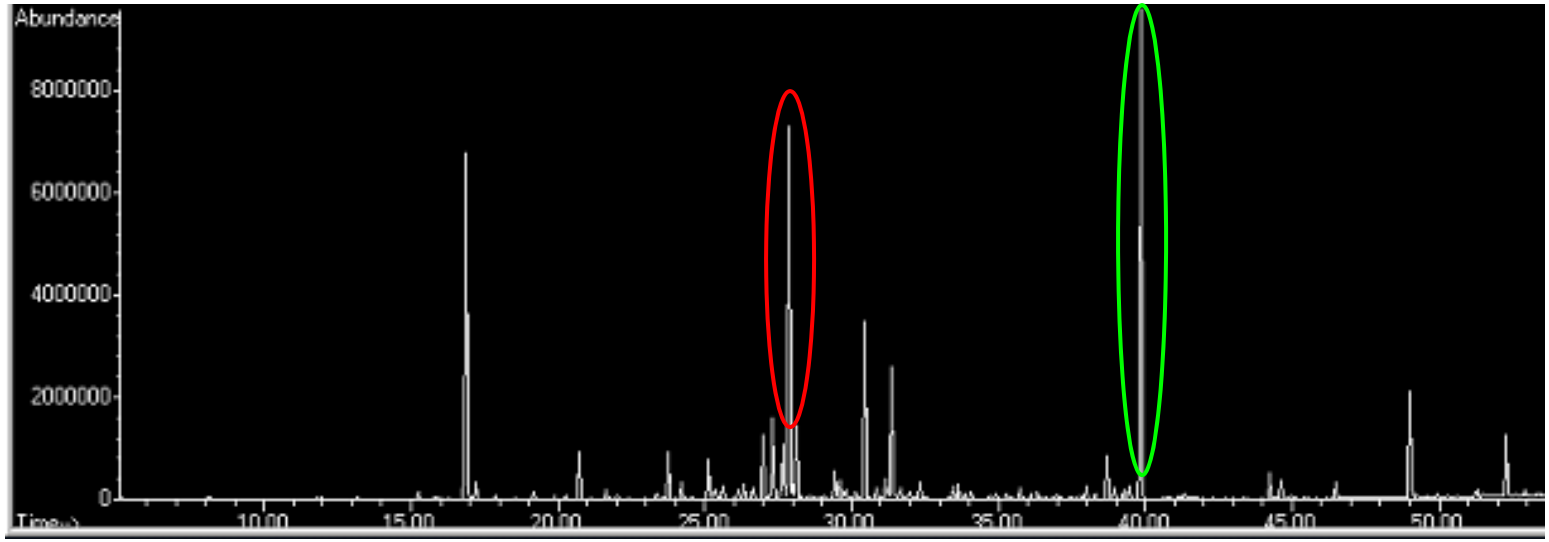


Metabolites Isolation and Derivatization

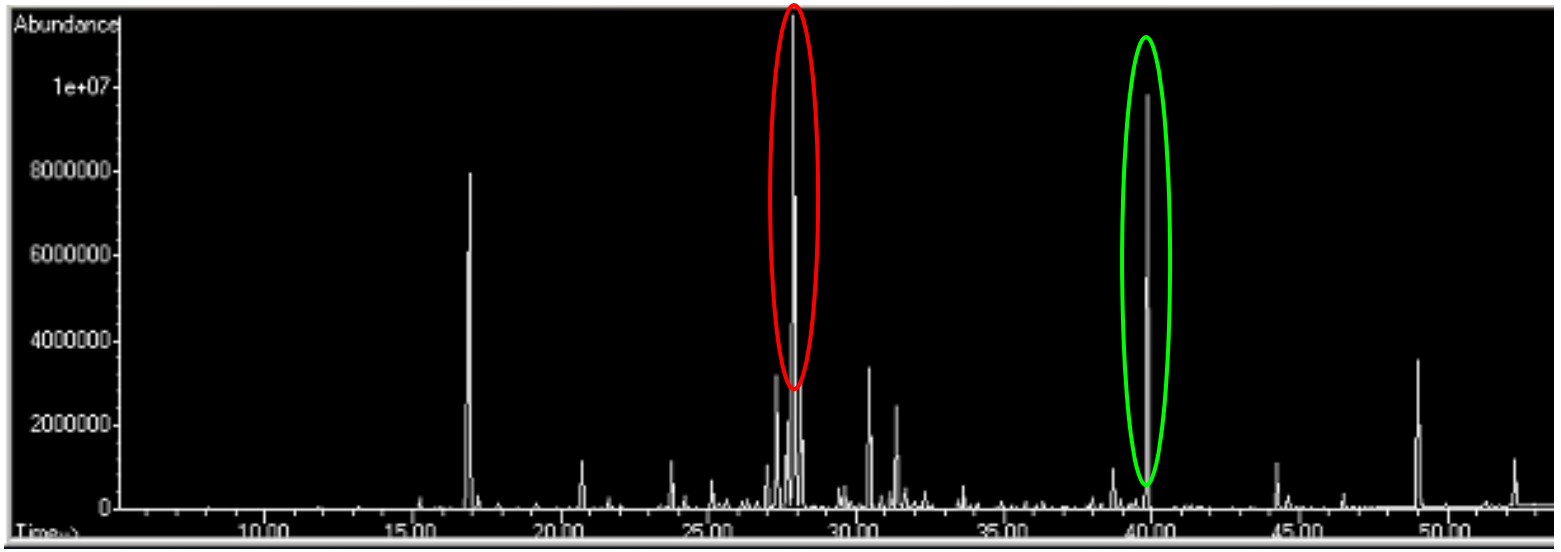


GC-MS captures metabolite profile in WT and *ukl2* mutant plants

WT



ukl2



Data Processing

Convert raw date file to Common Data Format file

↓

AMDIS_32: library search and metabolite identification for each peak

↓

MET-IDEA: metabolite quantification

↓

Total 288 metabolites were identified and quantified

Student t-test was performed to identify significant difference.

Table I. Partial Lists of the Metabolites Identified by Metabolite Profiling

	ukl2/Col	*p*
Amino acid:		
Glycine	1.04	0.042
L-Aspartic acid (3TMS)	3.16	0.032
L-Glutamic acid, N-acetyl-(2TMS)	0.56	0.001
L-Glutamic acid (3TMS)	1.40	0.046
L-Phenylalanine (2TMS)	1.43	0.049
Acids and hydroxy acids:		
Fumaric acid (2TMS)	1.35	0.008
Maleic acid (2TMS)	2.33	0.003
(R*,S*)-3,4-Dihydroxybutanoic acid (3TMS)	1.86	0.004
Threonic acid-1,4-lactone (2TMS), trans-	2.12	0.032
Malic acid (3TMS)	2.39	0.031
Picolinic acid (1TMS)	3.77	0.039
Erythronic acid (4TMS)	1.95	0.039
Propanoic acid, 2-oxo-3-(trimethylsilyl)- (3TMS)	1.35	0.027
Pentonic acid (5TMS)	1.40	0.035
Anthranilic acid	0.95	0.325
Benzoic acid, 3-(5-chloro-2-methoxybenzoyl)-4-hydroxy-	2.19	0.035
Dehydroascorbic acid dimer	4.27	0.005
Furo[2,3-H]coumarine	2.98	0.043
Benzoic acid, 2,6-bis(trimethylsiloxy)-, methyl ester	2.01	0.018
Hexadecanoic acid (1TMS)	1.68	0.003
9,12-Octadecadienoic acid	1.44	0.037
trans-Sinapinic acid (2TMS)	1.49	0.011
Butanoic acid	0.64	0.010
Sugars and sugar alcohols:		
Fructose methoxyamine {BP} (5TMS)	1.52	0.004
Psicose methoxyamine {BP} (5TMS)	2.16	0.001
Glucose methoxyamine (5TMS)	1.83	0.012
Glycerol(3TMS)	1.29	0.039
Sucrose (8TMS)	1.09	0.166
L-Mannose	1.56	0.008
Melezitose (11TMS)	2.28	0.033
Galactinol (9TMS)	2.35	0.035
Trehalose (8TMS)	1.01	0.478

Ion intensity was used to represent the compound abundance. Data is expressed as means ± standard error (n =5). Student t-test was conducted to calculate *p* value. The relative abundance in the *ukl2* and WT was represented by their mean ion intensity ratio (*ukl2/Col*).

Chen and Thelen (2011). Plant Cell 23:2991-3006.

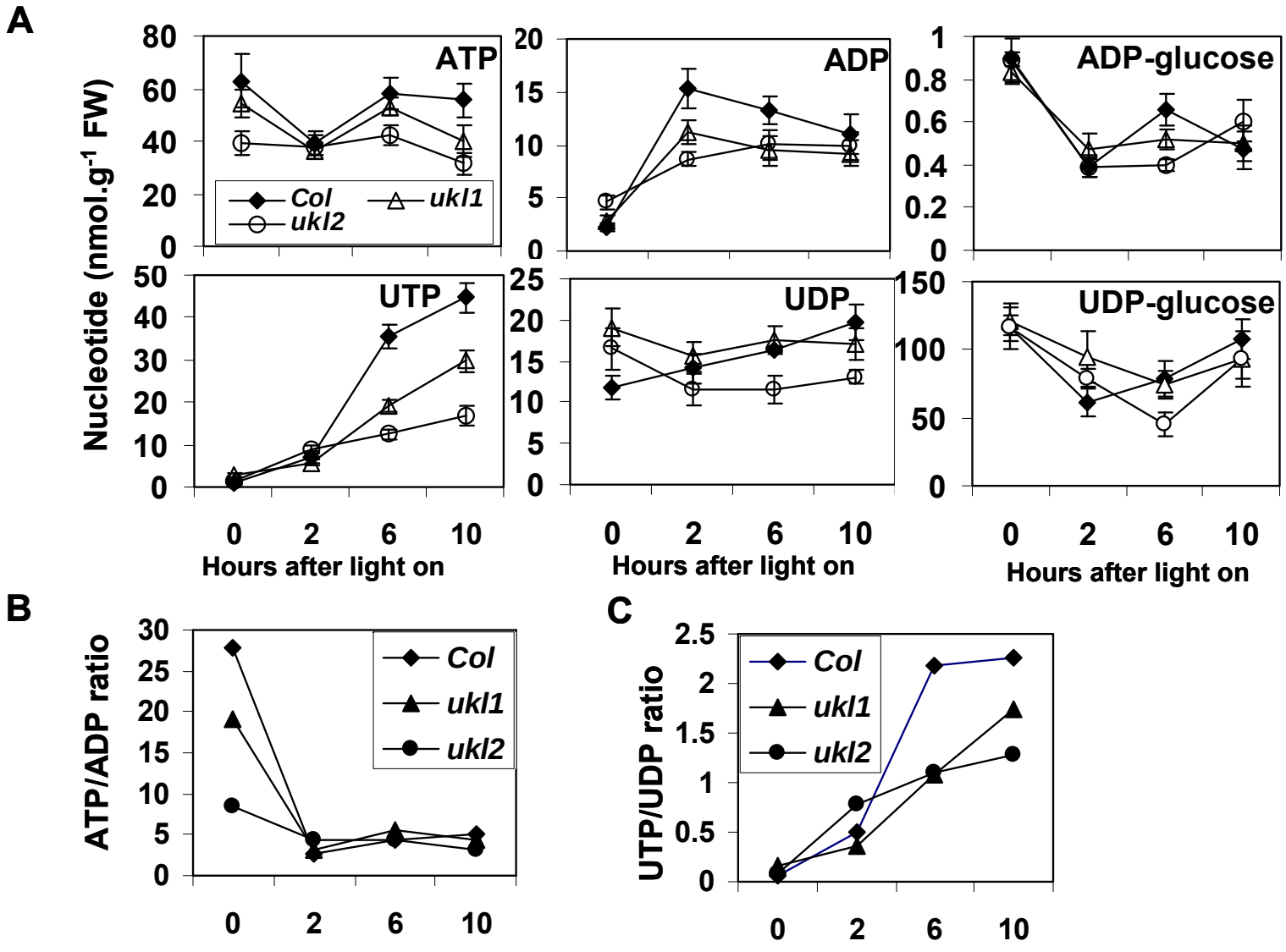
GC-MS profiled many metabolites but…

Nucleotides were not detected

Targeted nucleotide profiling by HPLC

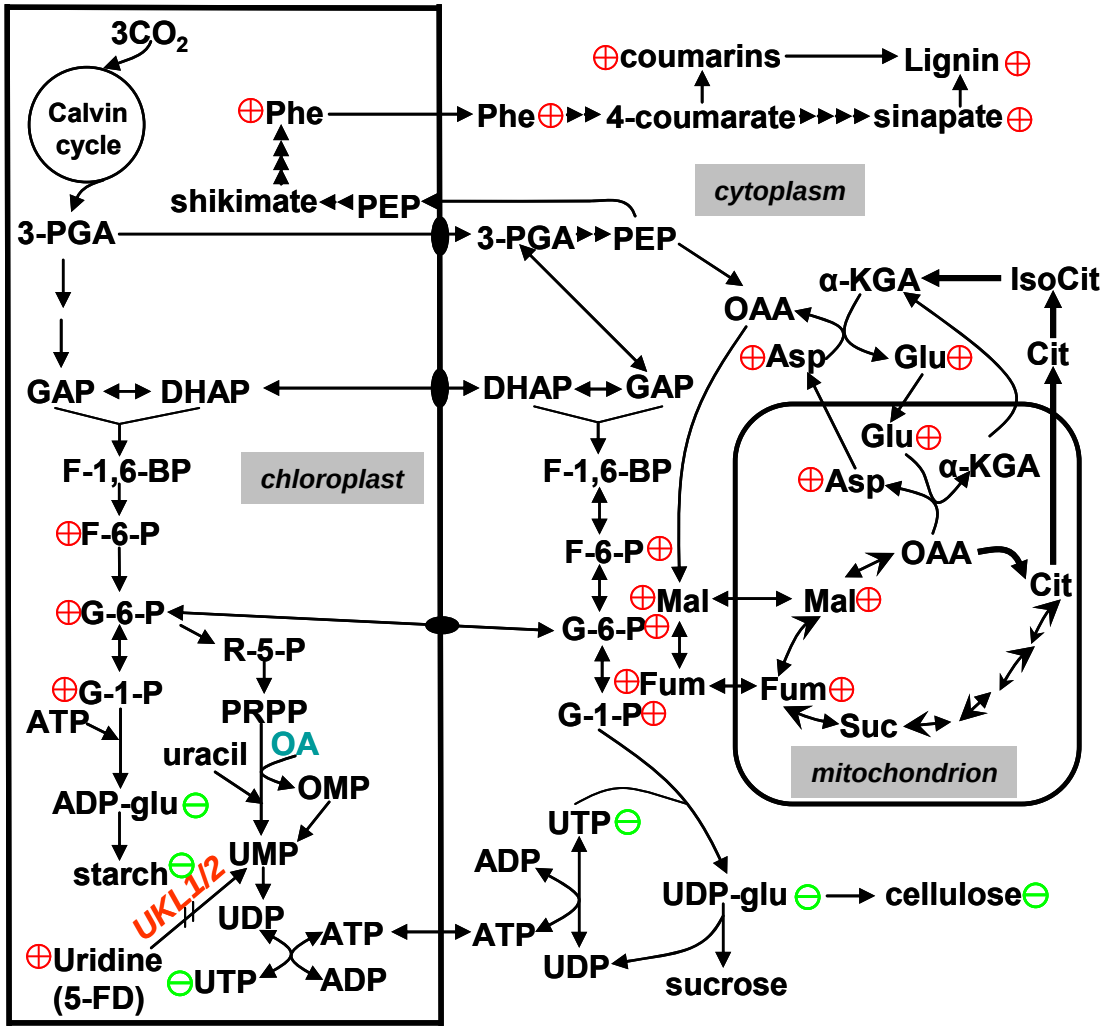
EC 250/4.6 nucleosil 100-10 SB column

Nucleotide profiling identified light-dependent alterations in *ukl2* mutant plants



Chen and Thelen (2011). Plant Cell 23:2991-3006.

A carbon flux model in *Arabidopsis* leaf



Chen and Thelen (2011). Plant Cell 23:2991-3006.

Review

Not just a circle: flux modes in the plant TCA cycle

Lee J. Sweetlove[1], Katherine F.M. Beard[1], Adriano Nunes-Nesi[2], Alisdair R. Fernie[2] and R. George Ratcliffe[1]

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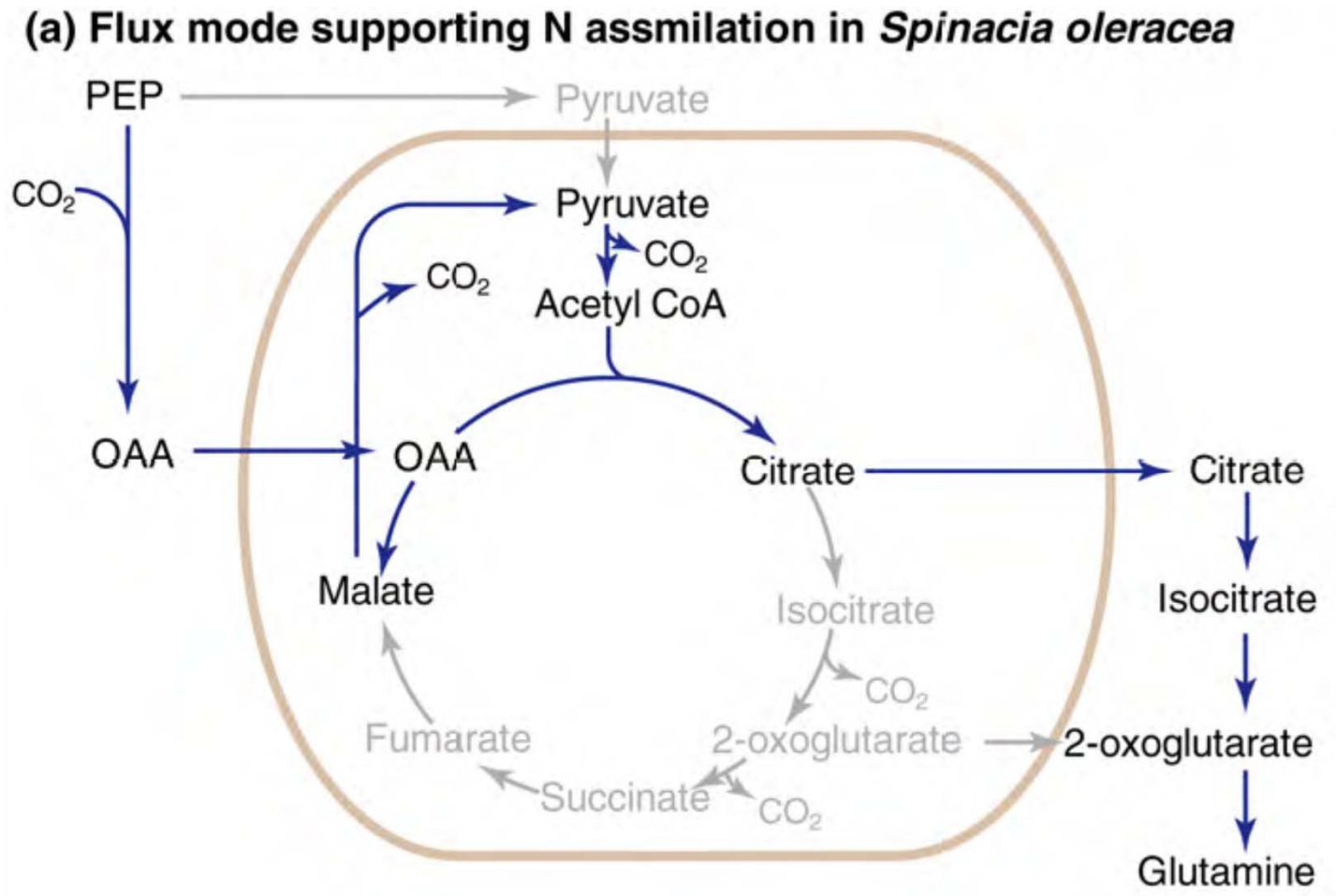
[2] Max-Planck Institute for Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany

The tricarboxylic acid (TCA) cycle is one of the iconic pathways in metabolism. The cycle is commonly thought of in terms of energy metabolism, being responsible for the oxidation of respiratory substrates to drive ATP synthesis. However, the reactions of carboxylic acid metabolism are embedded in a larger metabolic network and the conventional TCA cycle is only one way in which the component reactions can be organised. Recent evidence from labelling studies and metabolic network models suggest that the organisation of carboxylic acid metabolism in plants is highly dependent on the metabolic and physiological demands of the cell. Thus, alternative, non-cyclic flux modes occur in leaves in the light, in some developing oilseeds, and under specific physiological circumstances such as anoxia.

organic acids generated from other pathways, such as the glyoxylate cycle during lipid mobilisation [1–3]. These other functions require non-cyclic flux modes, and while they may or may not require an input of acetyl CoA, they all require an input of a TCA cycle intermediate to compensate for the loss of carbon from the cycle. This compensation is sometimes described as an anaplerotic process, but the use of this term is not necessary since maintaining the levels of intermediates is a fundamental requirement for any metabolic network in a steady state, not just the TCA cycle.

Although the operation of the cycle was demonstrated in plants decades ago [4], there remain many unanswered questions relating to the balance between cyclic and non-cyclic flux modes. The physiological context in which the pathway functions is an important factor in determining

Trends in Plant Science 15 (2010) 462-470



Hanning, I. and Heldt, H.W. (1993) On the function of mitochondrial metabolism during photosynthesis in spinach (Spinacia oleracea L.) leaves. Partitioning between respiration and export of redox equivalents and precursors for nitrate assimilation products. Plant Physiol 103, 1147–1154.

The Plant Cell, Vol. 23: 2991–3006, August 2011, www.plantcell.org

Plastid Uridine Salvage Activity Is Required for Photoassimilate Allocation and Partitioning in *Arabidopsis* [C][W]

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Nucleotides are synthesized from de novo and salvage pathways. To characterize the uridine salvage pathway, two genes, *UKL1* and *UKL2*, that tentatively encode uridine kinase (UK) and uracil phosphoribosyltransferase (UPRT) bifunctional enzymes were studied in *Arabidopsis thaliana*. T-DNA insertions in *UKL1* and *UKL2* reduced transcript expression and increased plant tolerance to toxic analogs 5-fluorouridine and 5-fluorouracil. Enzyme activity assays using purified recombinant proteins indicated that *UKL1* and *UKL2* have UK but not UPRT activity. Subcellular localization using a C-terminal enhanced yellow fluorescent protein fusion indicated that UKL1 and UKL2 localize to plastids. The *ukl2* mutant shows reduced transient leaf starch during the day. External application of orotate rescued this phenotype in *ukl2*, indicating pyrimidine pools are limiting for starch synthesis in *ukl2*. Intermediates for lignin synthesis were upregulated, and there was increased lignin and reduced cellulose content in the *ukl2* mutant. Levels of ATP, ADP, ADP-glucose, UTP, UDP, and UDP-glucose were altered in a light-dependent manner. Seed composition of the *ukl1* and *ukl2* mutants included lower oil and higher protein compared with the wild type. Unlike single gene mutants, the *ukl1 ukl2* double mutant has severe developmental defects and reduced biomass accumulation, indicating these enzymes catalyze redundant reactions. These findings point to crucial roles played by uridine salvage for photoassimilate allocation and partitioning.

INTRODUCTION

In higher plants, the net product of photosynthesis in a leaf that some key metabolites (e.g., orthophosphate, triose phosphate [cytosol-to-chloroplast concentration ratio], and fructose

Summary

- Metabolomics is an important technique with wide applications in biology
- Multiple analytical platforms are usually necessary to profile the large chemical diversity of metabolites in a cell (e.g. LC- & GC-MS)
- Metabolome alterations in *ukl2* mutants reflect the role of this enzyme in plant metabolism
- Pyrimidine salvage enzymes are required for fixed-carbon allocation and partitioning in leaves

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Thank You!!