How do cells respond to stress and developmental cues?

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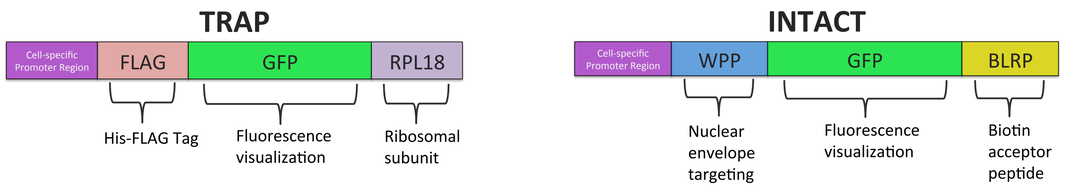
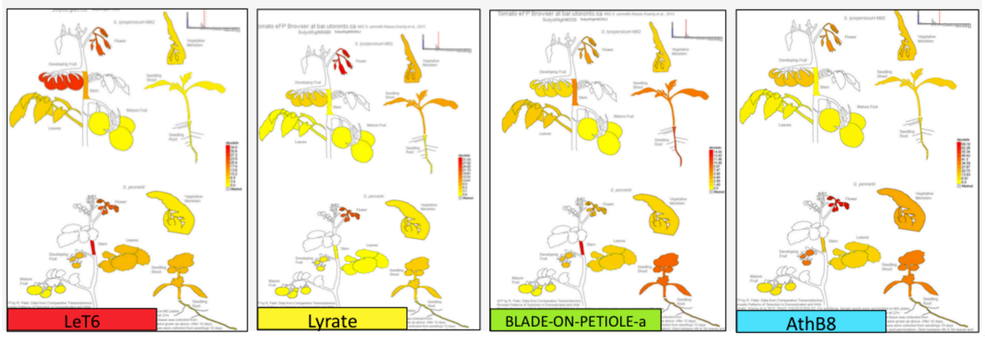
ABSTRACT: Leaf shape is tremendously diverse in nature, yet overall leaf function in photosynthesis is conserved through development. How the leaf responds to stress, hormonal, genetic, and environmental cues is relatively unexplored. To dissect molecular leaf development, cells transitioning between meristematic and leaf tissue types must be separated from homogeneous, lineage-independent clusters. Using Isolation of Nuclei TAgged in specific Cell Types (INTACT) and Translating Ribosome Affinity Purification (TRAP), cell-type specific molecular data can be isolated with confidence. In addition to discrete cell population differences, these techniques can be used in conjunction with water stresses to investigate developmental plasticity in a domesticated crop (*Solanum lycopersicum* var *M82;* tomato) and its desert, wild relative (*Solanum pennellii*). These studies will enable the identification of molecular events that determine leaf shape across two morphologically distinct *Solanum* species and those that determine their relative responses to water stress conditions.

Leaf Developmental Genetics via Cell Specificity



Key Leaf Patterning Genes

Expression patterns of four developmental genes in shoot tissues of tomato & *S. pennellii*



LeT6

Arabidopsis ortholog: SHOOT MERISTEMLESS (STM), a *KNOTTED-LIKE1 HOMEOBOX (KNOX)* gene
Expression Domain: central zone of the shoot apical meristem (SAM)
Possible Function: maintaining meristem identity

LYRATE (LYR)

Arabidopsis ortholog: JAGGED (JAG)
Expression Domain: in the SAM, incipient leaf primordia (P0), leaflet initiation sites
Possible Function: a *KNOX* repressor gene; allows organ initiation[1]

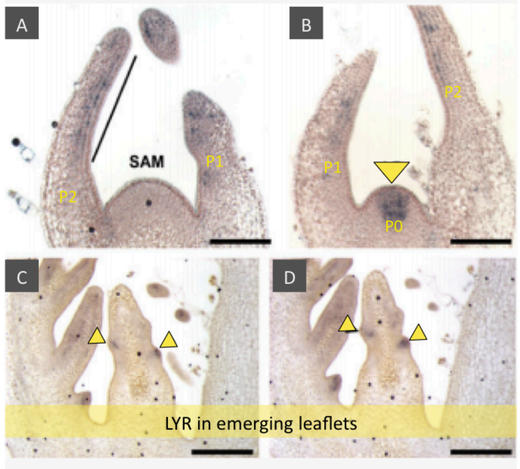
BLADE-ON-PETIOLE-a (BOPa)

Arabidopsis ortholog: BLADE-ON-PETIOLE-1
Expression Domain: boundary zones in leaf primordia[2]; mature leaf tissue mesophyll
Possible Function: repression of JAG; further organization of leaf tissue cell types

AthB8

Arabidopsis ortholog: ATHB8; class III homeodomain-leucine zipper (*HD-ZIPIII)*
Expression Domain: in leaf primordia, along vascular track, after auxin accumulation before differentiation
Possible Function: cell fate commitment to become vasculature tissue[3]

LYRATE in situ in Domesticated Tomato[1*]



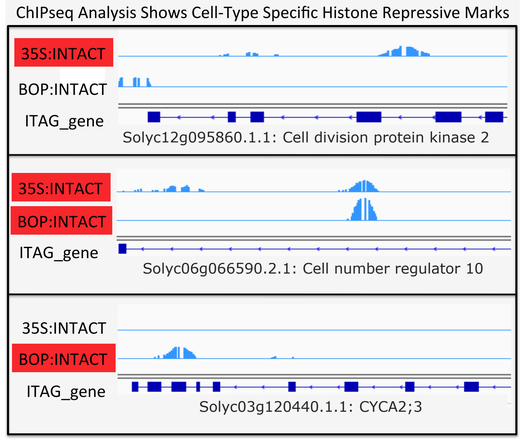
SAM = Shoot Apical Mersitem P0 = incipient primordium
P1-3 = older leaf primordia * altered from publication for clarity in this project

Planned Transgenic Lines for Leaf Development

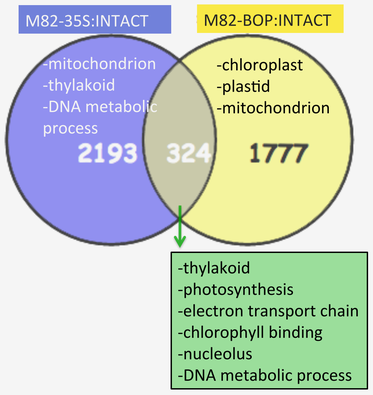
Solanum lycopersicum	INTACT - ChiP & Nuclear Transcriptome	LeT6
		LYR ✓
		BOPa ✓
		AthB8 ✓
	TRAP - Translatome & Ribosomal Footprints	LeT6
		LYR ✓
		BOPa ✓
		AthB8
Solanum pennellii	INTACT - ChiP & Nuclear Transcriptome	LeT6
		LYR ✓
		BOPa ✓
		AthB8
	TRAP - Translatome & Ribosomal Footprints	LeT6
		LYR
		BOPa
		AthB8

Preliminary ChIP seq Results: 35S vs BOPa in Mature Leaves

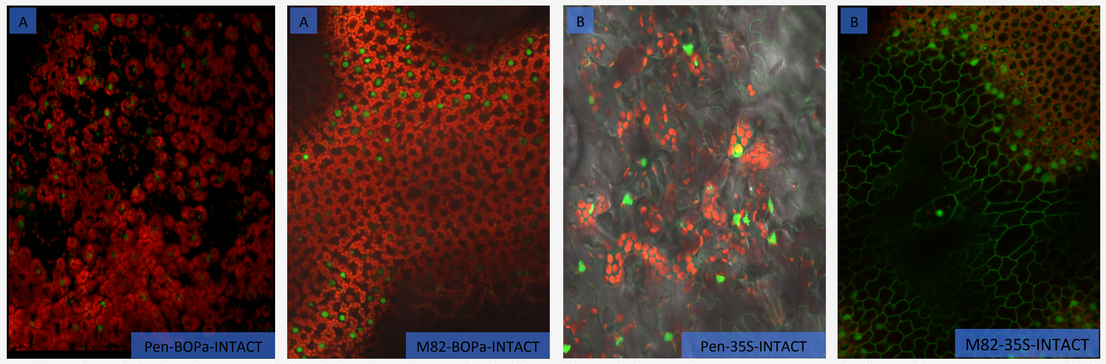
ChIPseq Analysis Shows Cell-Type Specific Histone Repressive Marks



Significant GO terms for Repressive Histone Enriched Peak Associated ITAGs



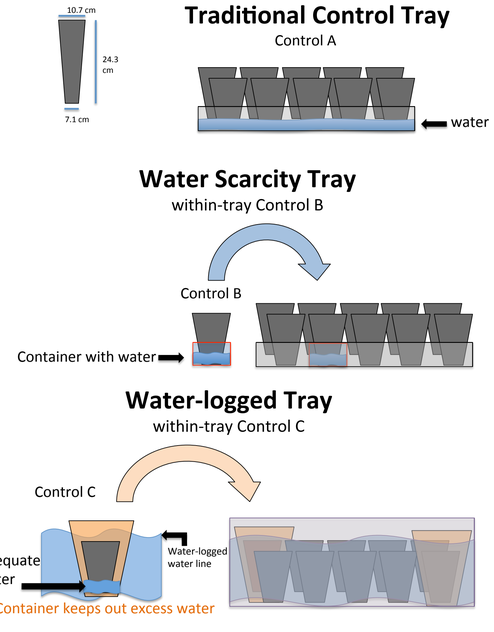
Confocal Images of Mature Leaves



A) GFP nuclei only localized to mesophyll or below B) GFP on nuclei highly visible in all observed vegetative tissue including trichomes, pavement cells & mesophyll. Images taken with LSM 710 laser scanning microscope (Carl Zeiss); 40x water immersion lens; Channels include chlorophyll A in red (647-721nm), GFP in green (495-528nm) and visible light spectrum (416nm-728nm)* to help identify tissue types (*Pen-35S-IN only)

Leaf Developmental Genetics under Water Stress

Once T3 lines are established, water stress experiments will be conducted over at least 3 biological replicates with 20 plants per replicate. In order to maintain growth conditions and minimize pest influences to compare between constructs, promoters and root & shoot systems, stress experiments will be conducted in growth chambers. Because positional effects have been demonstrated[4], within-tray controls will be utilized



Across Tissues & Labs

Reliable, speedy root extraction for both species is a requirement to compare root-specific promoters (Brady Lab) to shoot-specific promoters across tissue types under water stresses. Plants will thus be grown on Profile™, a clay bead substrate.

Higher Yield Root Extraction with Clay Bead Substrate

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