Multi-tier gene expression analyses of environmental plasticity: From nucleosomes to ribosomes in rice and other species

Mauricio Reynoso[a] , Germain Pauluzzi[a], Sean Cabanlit[a], Ralston Mataki[a], Kaisa Kajala[b], Donnelly West[b], Marko Bajic[c], Roger Deal[c], Siobhan Brady[b], Neelima Sinha[b], and Julia Bailey-Serres[a]

[a]Center for Plant Cell Biology, University of California, Riverside, CA 92521
[b]Department of Plant Biology, University of California, Davis, CA 95616
[c]Department of Biology, Emory University, Atlanta , GA 30322

NSF-PGRP-IOS 1238243

Contacts: PI Julia Bailey-Serres serres@ucr.edu; Co-PI Siobhan Brady sbrady@ucdavis.edu; Co-PI Neelima Sinha nrsinha@ucdavis.edu; Co-PI Roger Deal roger.deal@emory.edu

Abstract

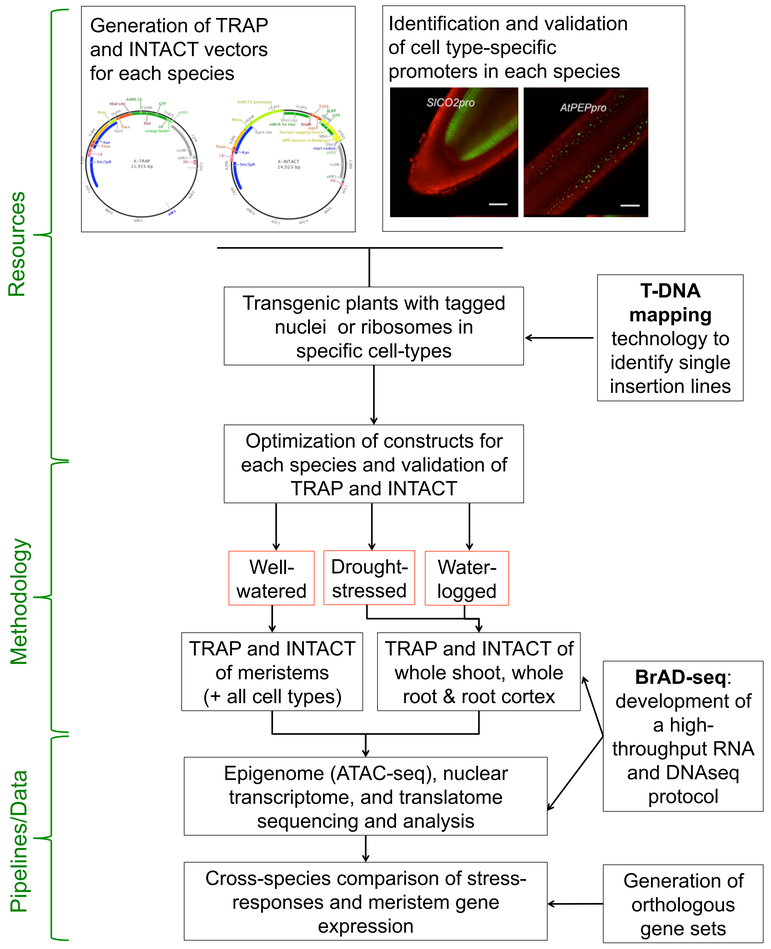
From seed germination to ovule fertilization, plant development is exquisitely orchestrated by genetic processes that are fine-tuned by environmental cues. This plasticity entails the precise regulation of networks of genes in individual cells. Of all the stresses experienced by crops, extremes in water are particularly damaging to yields. We are asking: **How does gene activity in stem cells (meristems) of roots and shoots differ across crop species? How do flooding and drought stress influence the development of specialized cell types in the root?**

To address these questions we have refined the **INTACT** (Isolation of nuclei tagged in specific cell types) and **TRAP** (Tagged ribosome affinity purification) technologies developed in Arabidopsis, which enable examination of the epigenome, transcriptome, and translatome of specific cell types.

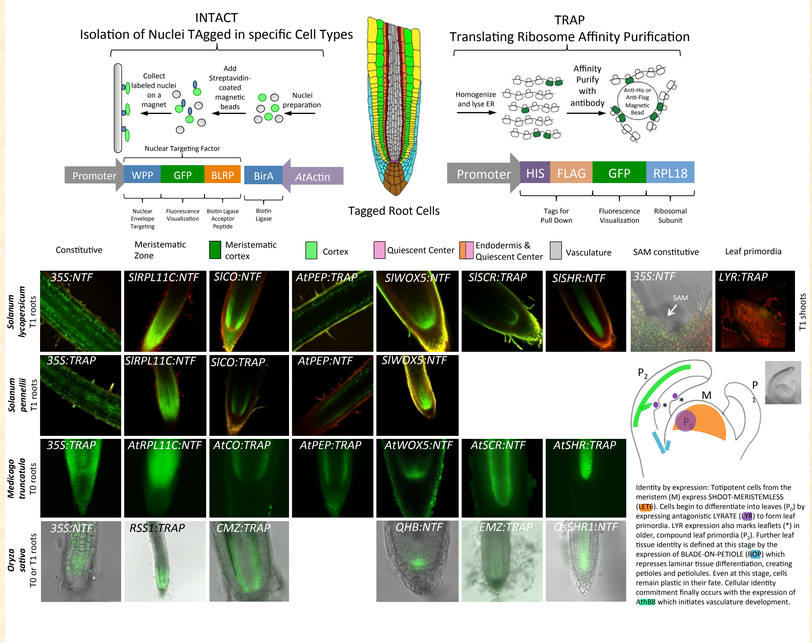
Our challenges and successes have been:

- To adapt INTACT and TRAP methods for crop species
- To establish *Agrobacterium rhizhogenes*-promoted hairy roots in tomato and Medicago
- To identify meristem and root cell-specific promoters
- To optimize INTACT in a monocot
- To integrate INTACT with "tagmentation" (ATAC-seq)
- To advance nuclear RNA, mRNA and ribosome footprint library construction with limited rRNA contamination
- To establish pipelines for data analysis

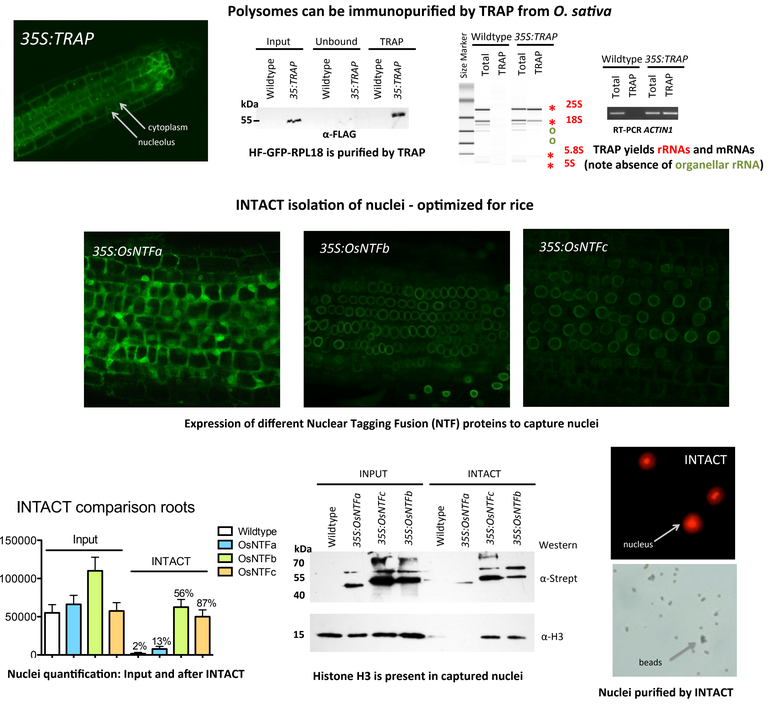
1. Project Workflow



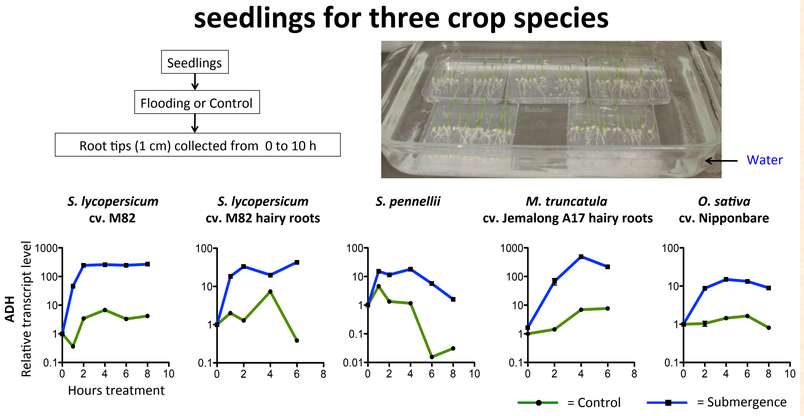
2. Establishment of INTACT and TRAP lines in four species



3. Purification of nuclei by INTACT and polysomes by TRAP as illustrated in rice

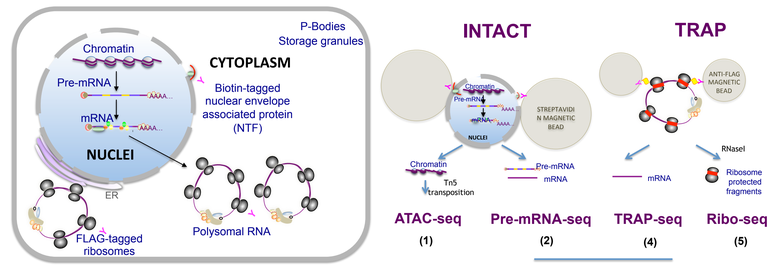


4. Validation of common submergence conditions in seedlings for three crop species

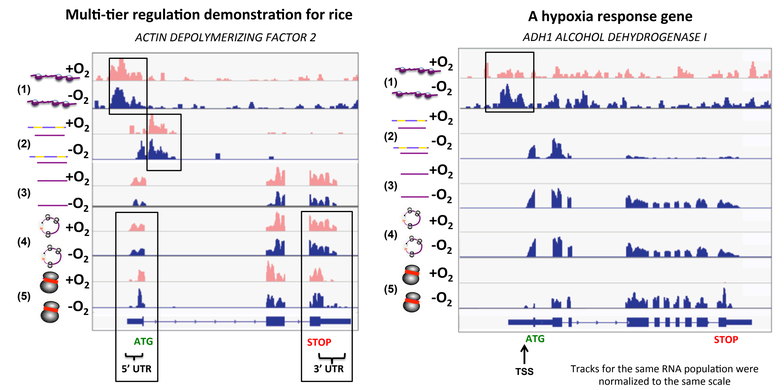


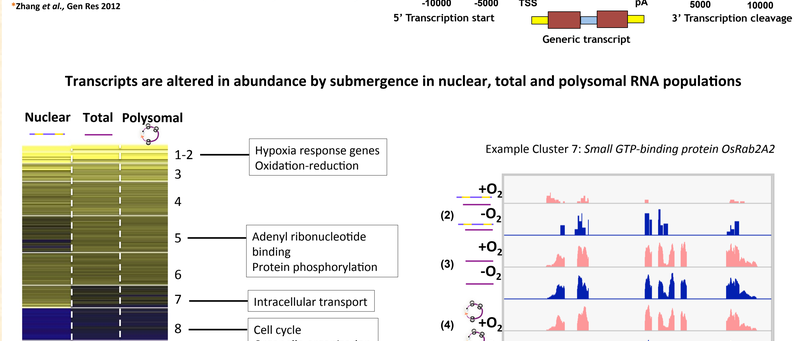
Alcohol dehydrogenase (*ADH*) mRNA levels, encoding an enzyme required for anaerobic metabolism, were quantified by qRT-PCR. Values were normalized to *ACT2* mRNA levels for *S. lycopersicum* and *S. penelli, RPL2* for *M. truncatula,* and *UBCII* for *O. sativa.* **The 2 hour time point was selected for inter-species comparison** of nuclear transcriptome, translatome and chromatin regulation by the stress.

5. Chromatin, nuclear pre-mRNA and polysomal mRNA analyses in root meristematic regions under submergence



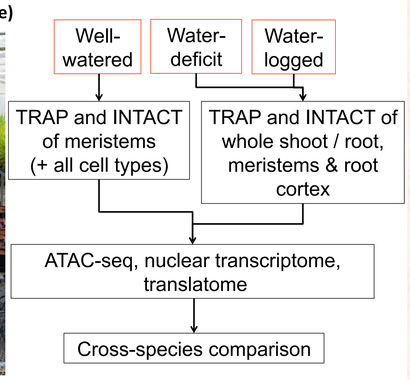
Annu. Rev. Plant Biol **Bailey-Serres 2013**





6. Current work of the Plasticity Project group

(1) Cell-type specific atlas under water stress conditions in the greenhouse (illustrated for rice)



(2) Field validation of new technologies for rice, tomato and medicago at UCR and UCD