ACCESSING MULTI-TIERED AND CELL-SPECIFIC REGULATION OF RESPONSES TO WATER EXTREMES IN RICE

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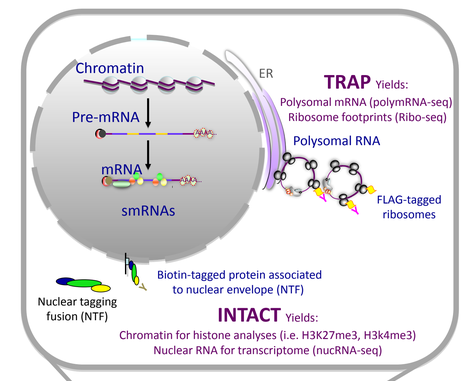
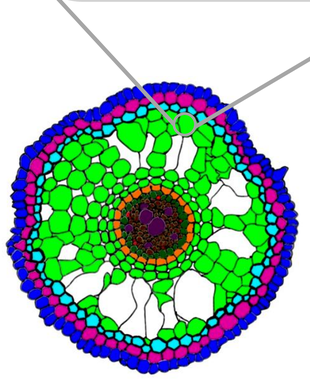
Floods and droughts are increasingly experienced in agricultural systems worldwide. In rice (*Oryza sativa* L.), these extremes in water availability invoke responses at the organ-, tissue- and cell-specific levels that enable metabolic to developmental responses relevant to survival. For example, the *SUB1A* gene is induced by submergence in meristematic regions. It confers tolerance to complete submergence by limiting growth, reducing cell damage and, protecting axillary shoot meristem viability (1). In the root system, specific cell types play varied roles under different water availability conditions. As an example, several layers of cortex differentiate in lysigenous cortical aerenchyma. These large lacunae serve as gas conduits from aerial tissue to the root and alleviate oxygen deprivation in waterlogged and compact soils. Furthermore, a layer of exodermis and sclerenchyma situated between the epidermis and the cortex are critical in limiting radial oxygen loss and water stress. Our project goal is to assess different layers of gene regulation of specific cell-types during development and in response to extremes in water availability. To achieve this goal we have established technologies that allow access to the epigenetics modifications (epigenome), nuclear transcriptome and ribosome-loaded transcripts (translatome) of target cell-types in rice. Isolation of Nuclei TAgged in specific Cell Types (INTACT) (2) has been transferred to rice to affinity purify nuclei, survey chromatin (*i.e.*, histone modifications) and quantify nuclear RNA populations. Translating Ribosome Affinity Purification (TRAP) (3) has been established in rice to immunopurify ribosomes and associated mRNAs. As a proof-of-concept we employed both technologies with near-constitutive and cell-type specific promoters to drive expression of the fusion proteins. For INTACT we used the WPP1 nuclear envelope targeting sequence-GFP-biotin ligase recognition peptide fusion construct developed for Arabidopsis. The biotin ligase protein was codon optimized for rice. For TRAP we used a His-FLAG-GFP-tagged version of a rice ribosomal protein L18 (RPL18). The effectiveness of these reagents in transgenic rice was compared to *Agrobacterium rhizogenes*-transformed tomato roots and transgenic Arabidopsis seedlings. We have characterized a number of root cell-specific and meristematic promoters from rice for our collaborative cross-species analysis of water stress responses in rice, tomato and Medicago.

***SUB1A* is expressed in shoot basal meristem.**

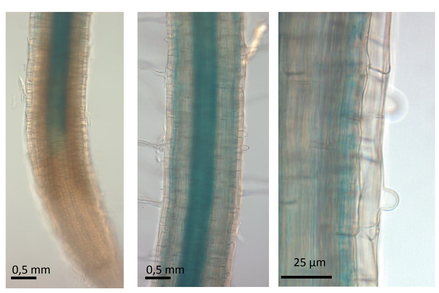
Cell-type Systems Approach





Root cell-type specific promoters

Root Meristem — Root Stele



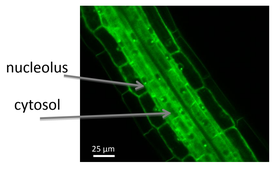
Exodermis / Endodermis

GUS assay on T0 regenerated seedlings of cell-type specific promoter candidates.

Translating Ribosome Affinity Purification (TRAP)

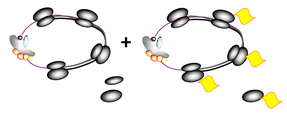
Agrobacterium tumefaciens transformation of rice calli

Proof-of-concept using 35S Promoter

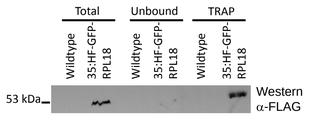


35S:HF-GFP-RPL18 transgenic roots

TRAP is feasible in *O. sativa*

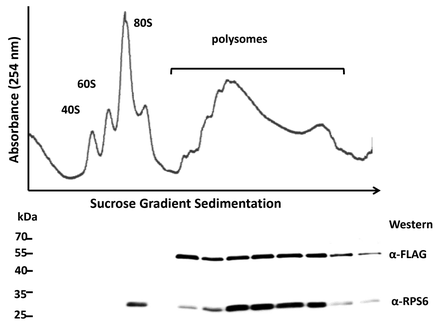


FLAG-tagged ribosomes can be immunopurified

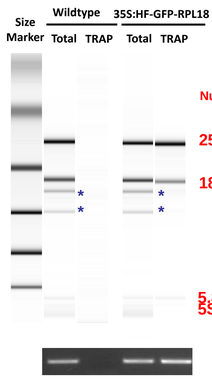


HF-GFP-RPL18 is present in TRAP sample

HF-GFP-RPL18 is incorporated into polysomes



TRAP yields rRNAs and mRNAs



Agilent 2100 Analysis of RNA samples. Total RNA and TRAP polysomal RNA from leaves of Rice wild type and expressing HF-GFP-RPL18.

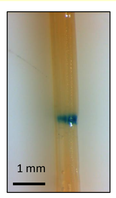
* Plastid rRNA?

RT-PCR *ACTIN1*

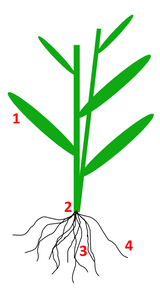
ACTIN1 mRNA is detected in TRAP sample

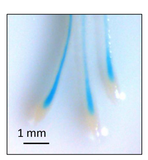
TRAP samples yield HF-GFP-RPL18, which is incorporated into polysomes, ribosomal RNA (rRNAs) and mRNAs associated with these complexes involved in translation

Cell-type specific TRAP using *OsSHR1* promoter

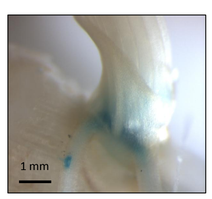


1 *OsSHR1* expressed in leaf lamina joint not leaf blade. TRAP done with leaf blade only.

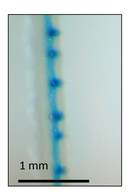




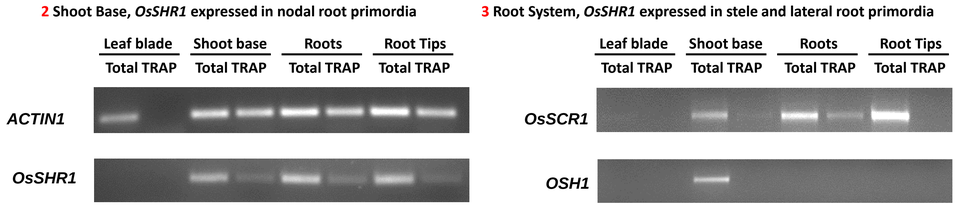
4: Root Tip, Stele specific



2 Shoot Base, *OsSHR1* expressed in nodal root primordia

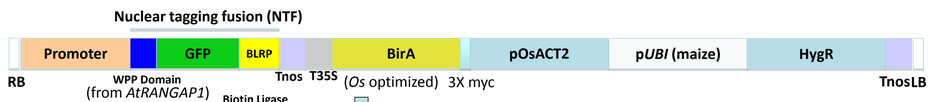


3 Root System, *OsSHR1* expressed in stele and lateral root primordia



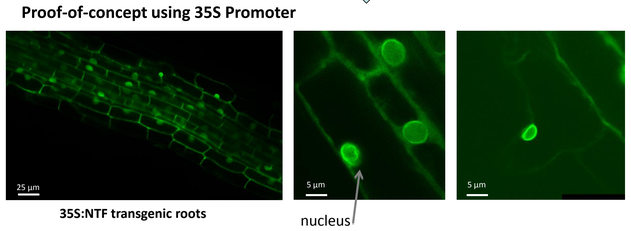
Cell Type specific polysomes immunopurification shown by *ACTIN1, SHR1, SCR1* and *OSH1* mRNA amplification by endpoint PCR. The absence of detection of *OsSCR1* in TRAP fraction of root tips and of *OSH1* in TRAP fraction of shoot base confirm the cell-specificity of the mRNAs

Isolation of Nuclei TAgged in specific Cell Types (INTACT)



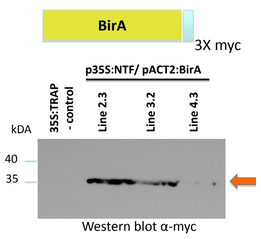
Agrobacterium tumefaciens transformation of rice calli

Proof-of-concept using 35S Promoter



35S:NTF transgenic roots

Optimized Biotin Ligase BirA is expressed in transgenic rice



Western blot α-myc

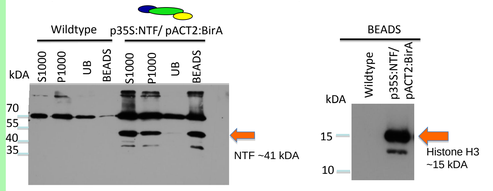
INTACT yields nuclei in *O. sativa*

Transgenic tissue promoter:NTF ACT2:BirA → Crude Nuclei preparation → Binding to Streptavidin Dynabeads → Nuclei Quantification Extraction of DNA, RNA or Proteins

NTF is present in crude and affinity purified nuclear fractions

Histone H3 is present in affinity purified nuclear fractions

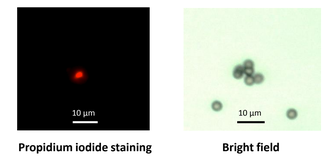
mRNA is detected on nuclear fractions



Streptavidin Western Blot — Western blot α-Histone H3 — RT-PCR for *GADPH*

S1000 Supernatant of the crude nuclei preparation
P1000 Crude nuclei extract
UB Unbound fraction
BEADS INTACT fraction bound to Streptavidin Dynabeads

Nuclei visualization

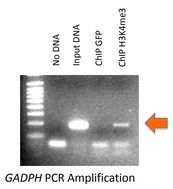


Propidium iodide staining — Bright field

Nuclei yield is estimated in about 350,000 nuclei per gram of leaves tissue

Chromatin Immunopurification (ChIP) is feasible using INTACT purified rice nuclei

35S:NTF ACT2:BirA leaves treated with formaldehyde → INTACT → ~100,000 nuclei as input → α- H3K4me3 0.5 ug, α- GFP 0.6 ug (neg. control)



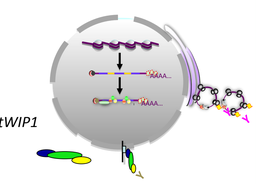
GADPH PCR Amplification

GADPH gene is amplified from ChIP DNA

Can nuclei yield be improved for rice?

Replacement of WPP domain with *O. sativa* homologue to *AtRANGAP1*

Design of GFP fusion protein containing *O. sativa* homologue to *AtWIP1 (WPP interacting protein)* containing a transmembrane domain



FUTURE WORK

- **Generation of rice transgenic lines expressing NTF and HF-GFP-RPL18 under cell-type specific promoters**
- **Drought and submergence treatments on TRAP and INTACT lines**
- **Translatome, epigenome and nuclear transcriptome for systematic comparison with tomato and Medicago**
- **Identification of genetic components that specify exodermis, sclerenchyma and Cortex**

References & Acknowledgements

1) Bailey Serres *et al* (2012). *Trends in Plant Sci* 17, 130.
2) Deal *et al* (2010). *Nature Prot* 6, 56.
3) Zanetti *et al* (2005). *Plant Phys* 138, 624

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