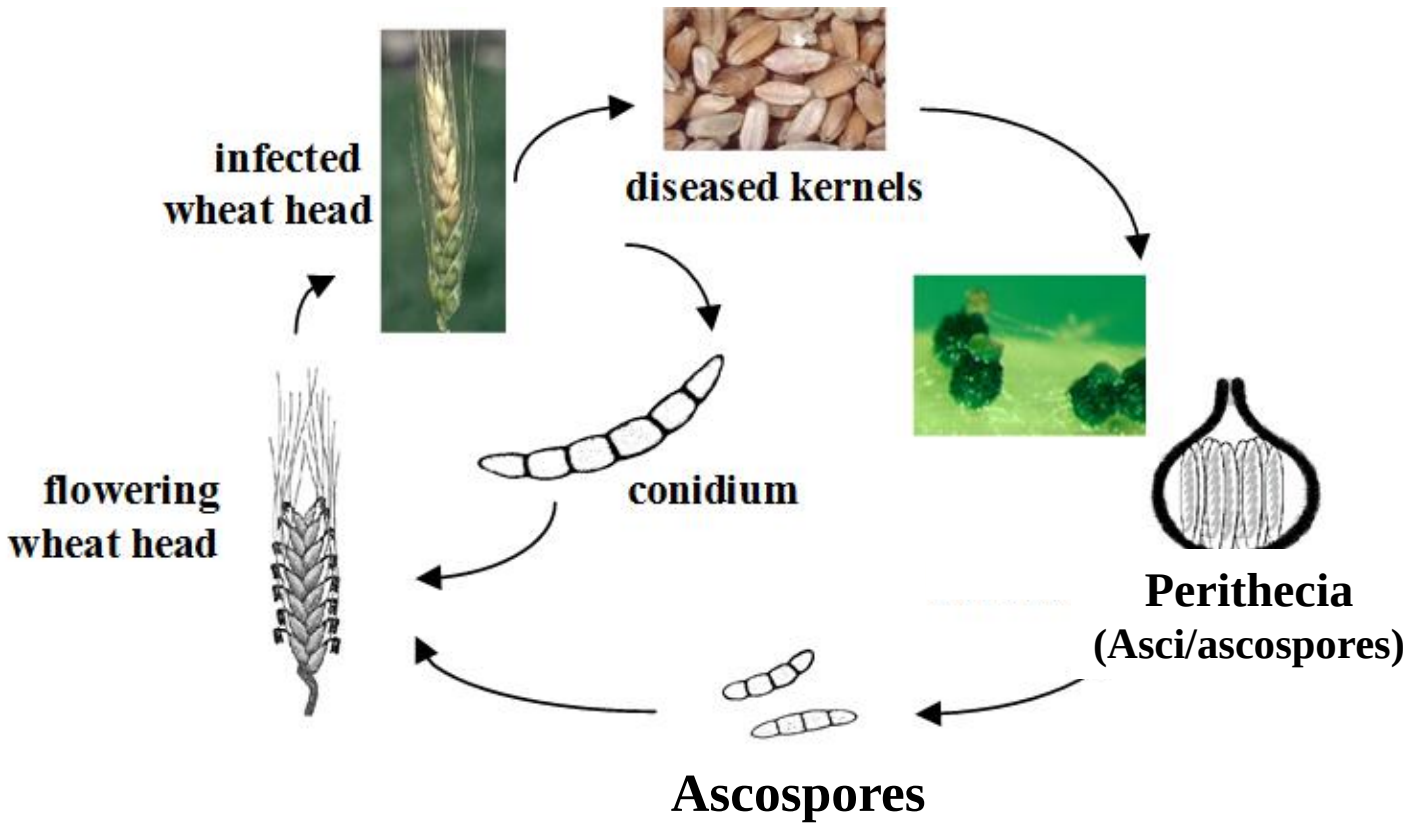
Orphan proteins of *Fusarium graminearum* important for wheat infection

Jin-Rong Xu

Purdue University

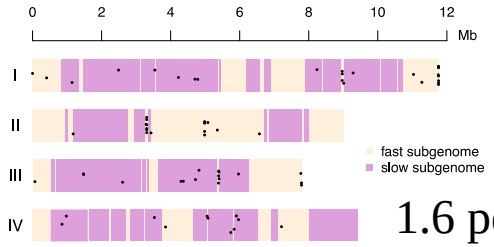
Scab or head blight (FHB) of wheat & barley



- **Deoxynivalenol (DON)**
- **Sexual reproduction plays a critical role in the disease cycle**

- **Regulation of DON biosynthesis**
  * antisense and lnc-RNA transcripts of *TRI5* and *TRI6*
  * ammonium suppression (Jiang et al., 2020. PLoS Genetics)

- **Contribution of elevated mutation rate during meiosis**
  Genetic variations – sexual reproduction

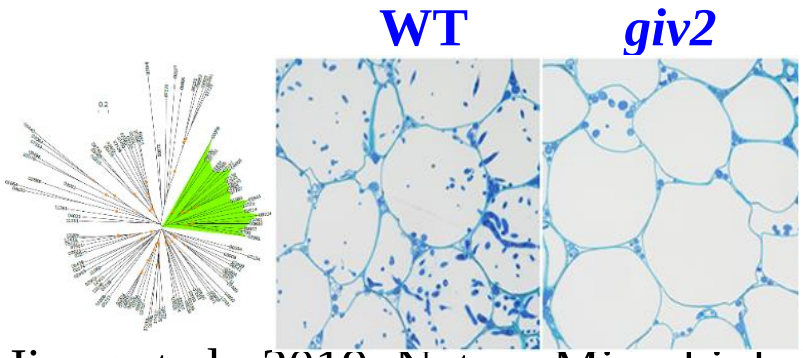


-homothallic (selfing), haploid fungus
-mutations during the repairing of DSBs

1.6 per meiosis

(Wang et al., in preparation)

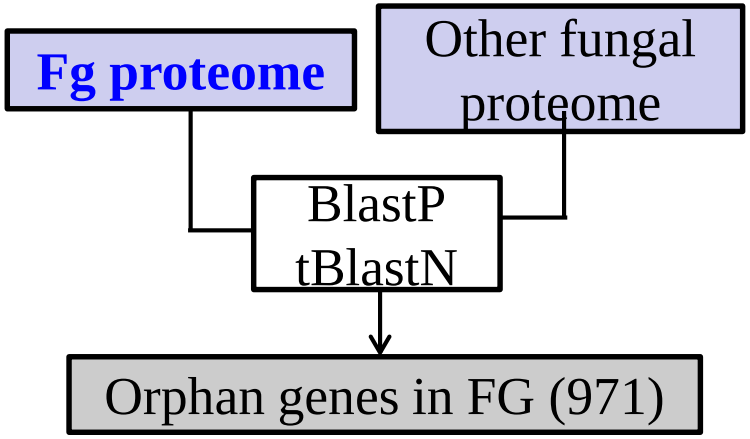
- **G-protein coupled receptors (GPCRs)**
  *recognizing stage/tissue-specific ligands



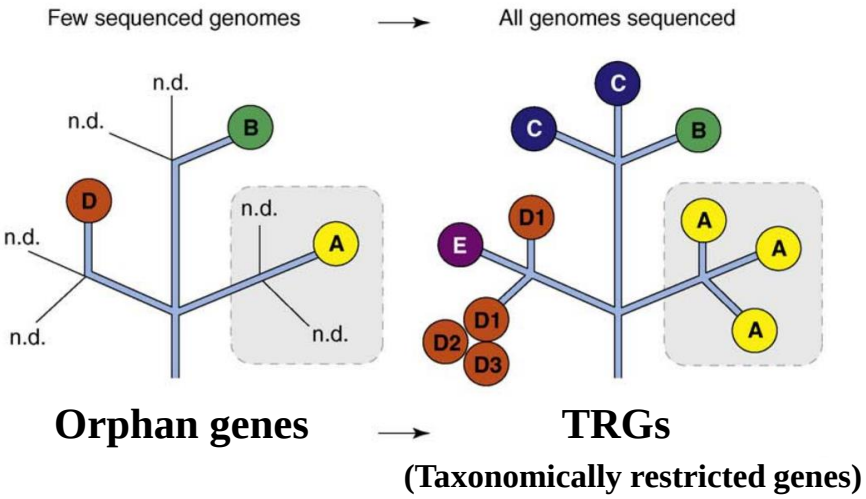
Jiang et al., 2019, Nature Microbiology

Orphan genes of *Fusarium graminearum*

-Restricted to a single species or narrow clade
-Often have unknown functions
-May be important for lineage-specific adaptations

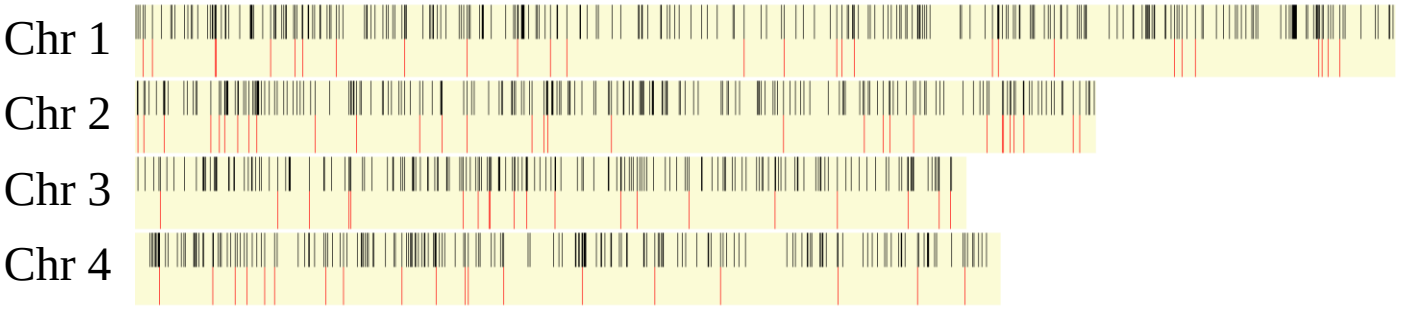


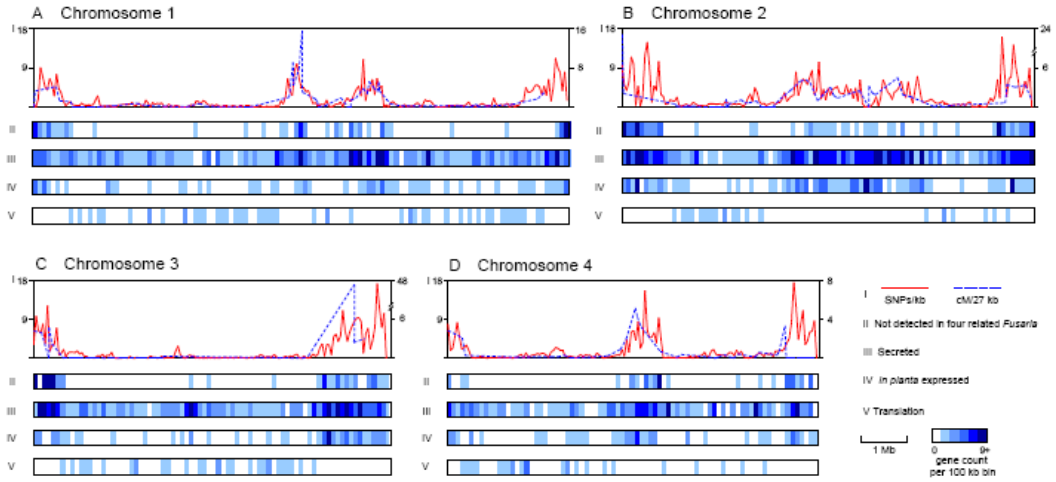
- 7.3% of protein encoding genes
- Shorter protein length
- Less transcribed



(Khalturin et al., 2009)

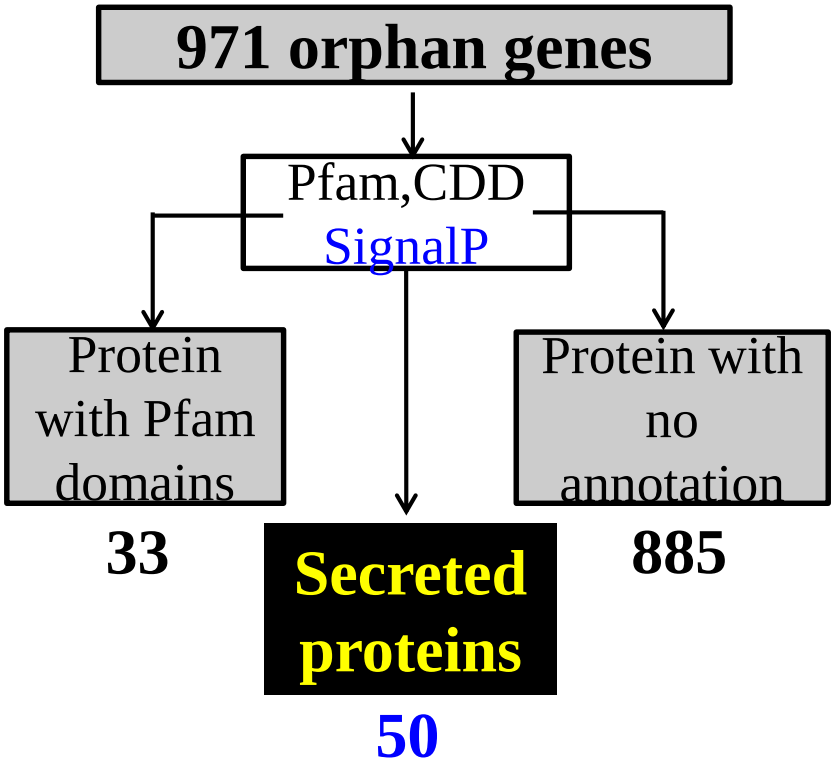
971 orphan genes in *F. graminearum*

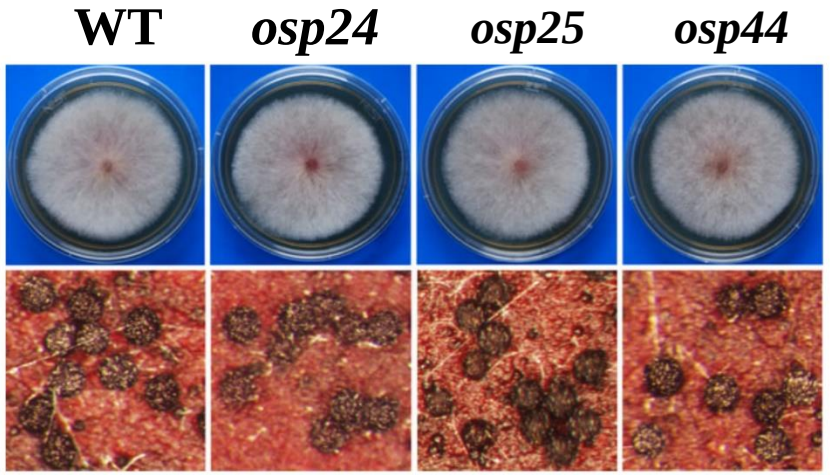




(Cuomo et al., 2007)

Orphan secretory protein (*OSP*) genes

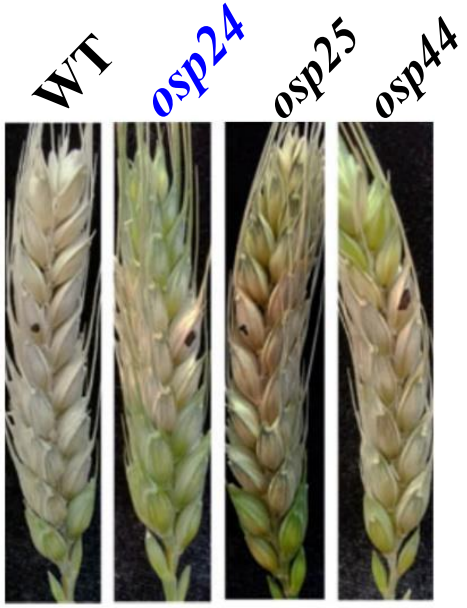


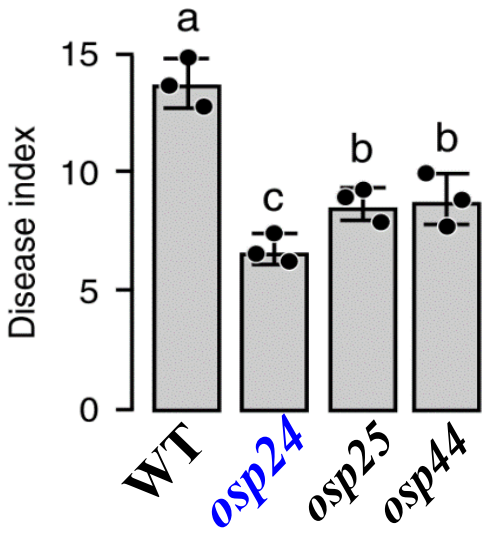


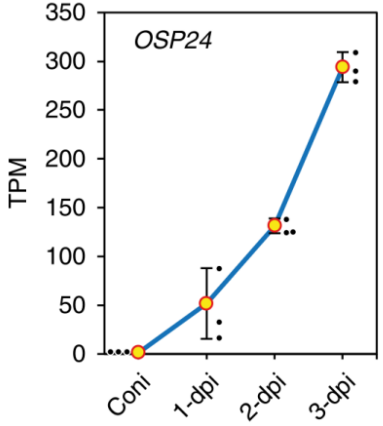
All the 50 *osp* deletion mutants were normal in growth and sexual/asexual reproduction

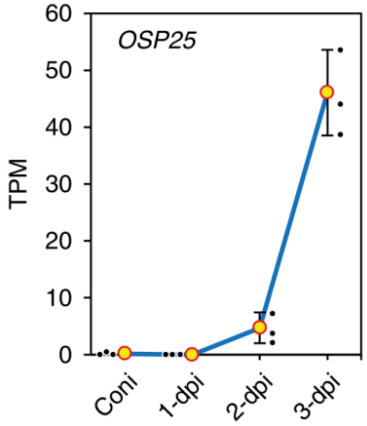
Jiang et al., 2020. Nature Communications

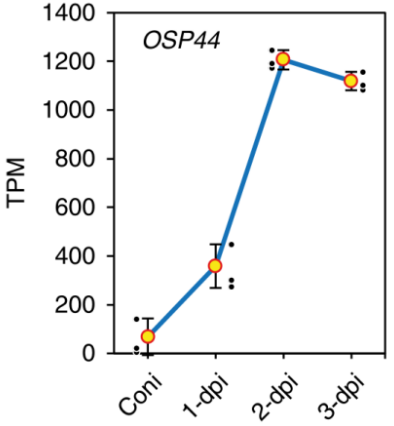
Three *OSP* genes are important for virulence





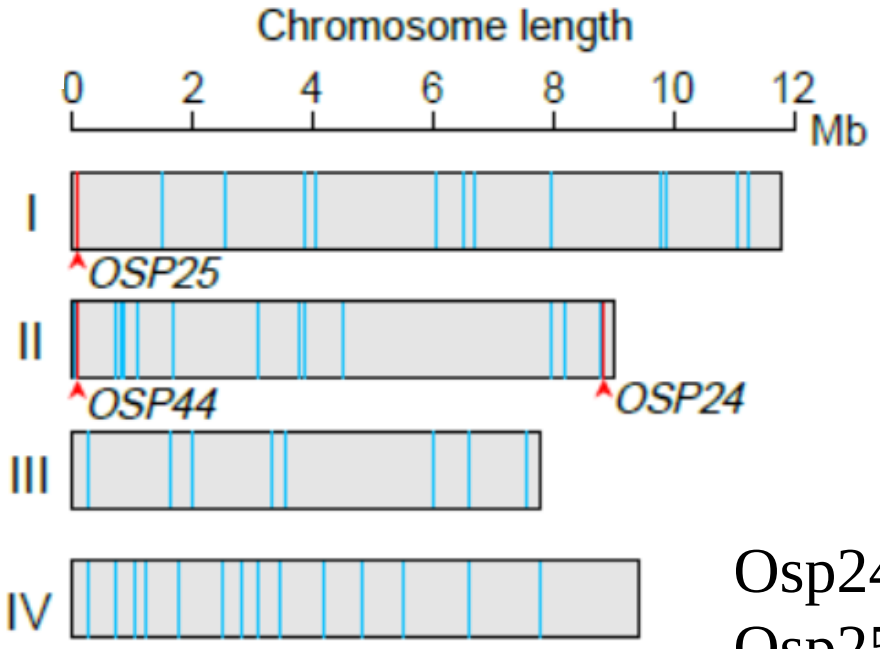






Highly up-regulated during infection

OSP24, *OSP25*, and *OSP44* - near the telomeric region
- small, cysteine-rich proteins



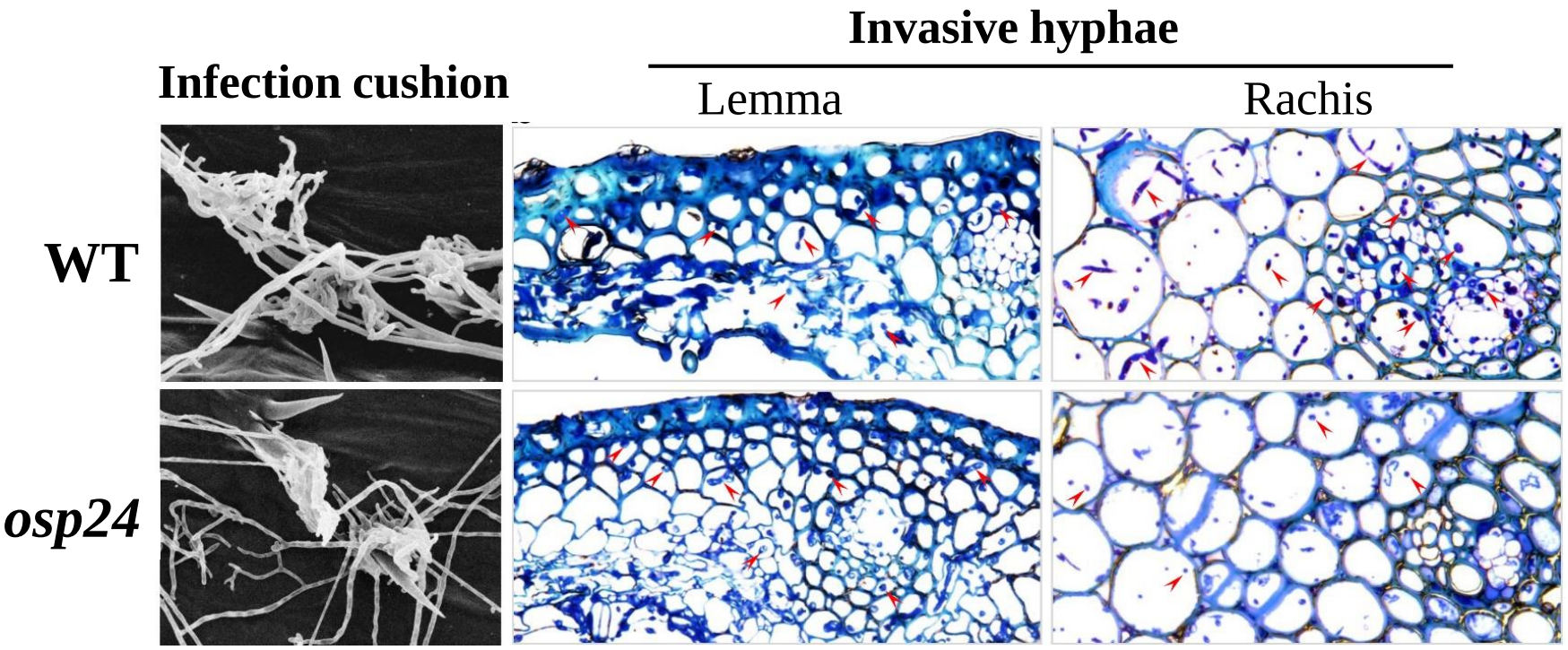
Osp24: 136 aa, 8C
Osp25: 116 aa, 8C
Osp44: 90 aa, 8C

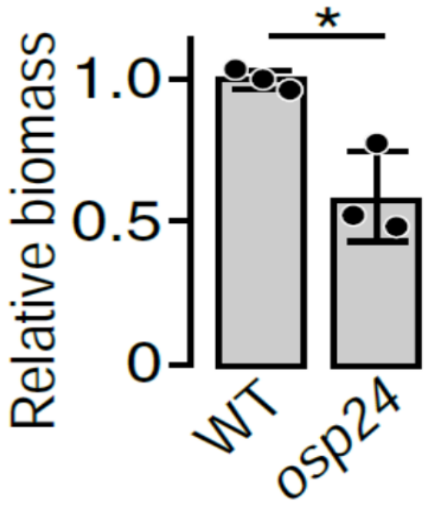
Effector genes in *Magnaporthe oryzae*

Peng et al., 2019. PLoS Genetics

Ma and Xu, 2019. PLoS Genetics

OSP24 is important for infectious growth in the rachis

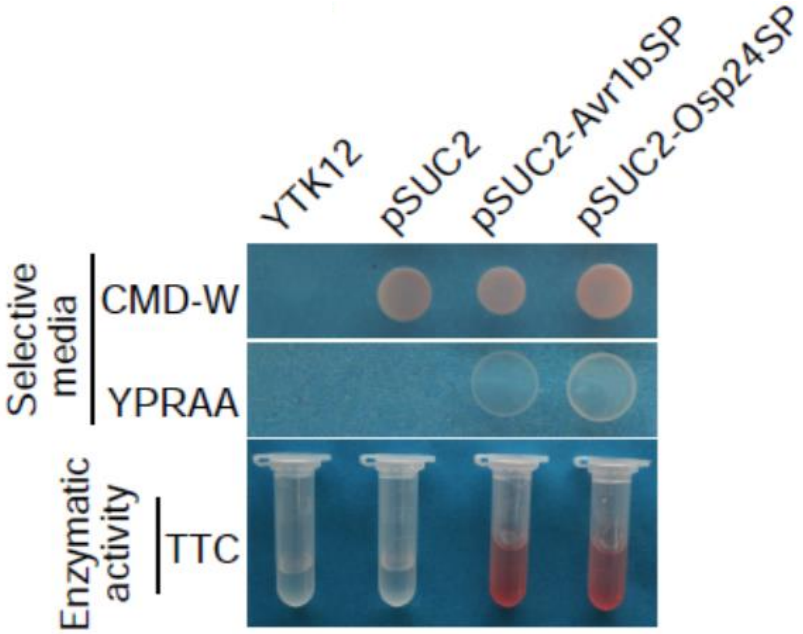




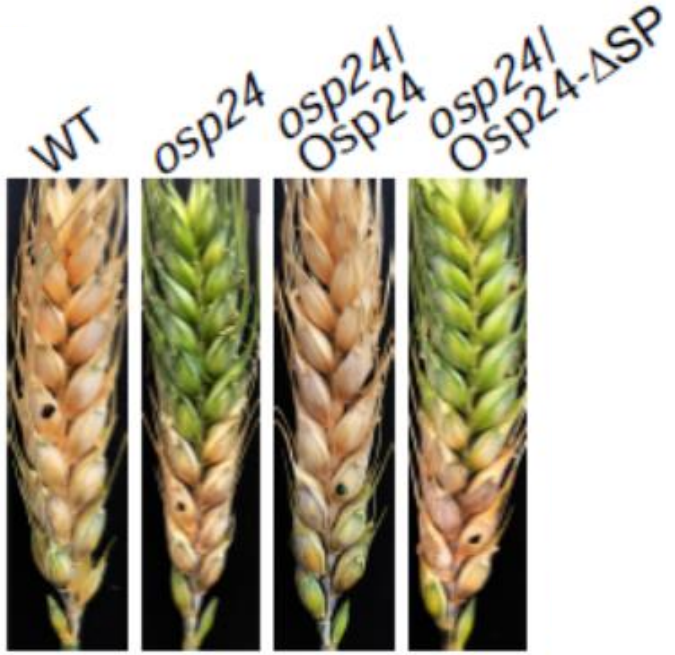
The *osp24* mutant is reduced in infectious growth

Signal peptide is required for secretion and function of Osp24

SP^{Osp24}-yeast *SUC2* invertase



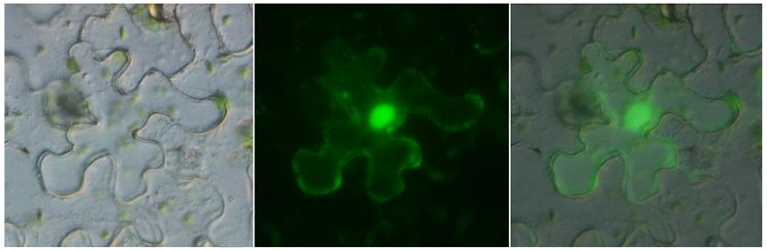
Signal peptide of Osp24 is functional in yeast

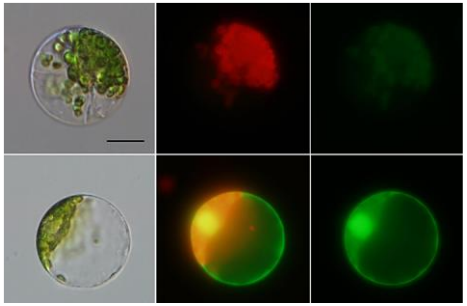


SP^{Osp24} is essential for its function

- No predicted NLS

Localization of Osp24 to the nucleus in plant cells

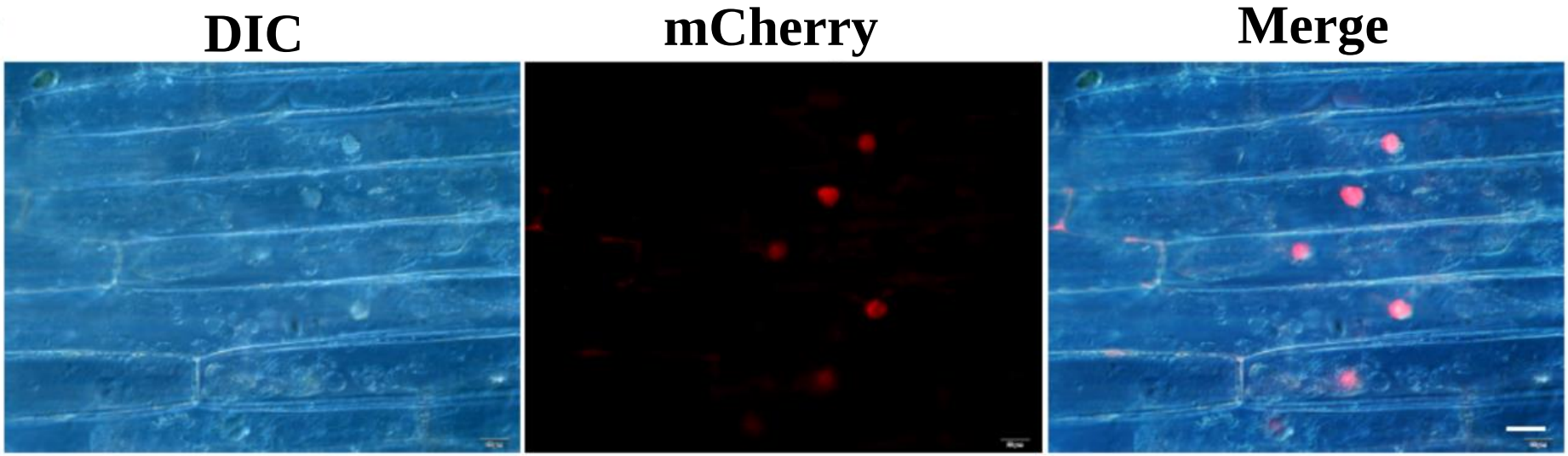




Transient expression of *OSP24*-GFP in *Nicotiana benthamiana*

Osp24 is a cytoplasmic effector

(It may be translocated into plant cells ahead of invasive growth)

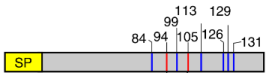


- Wheat coleoptile cells

- The *OSP24*-mCherry transformant

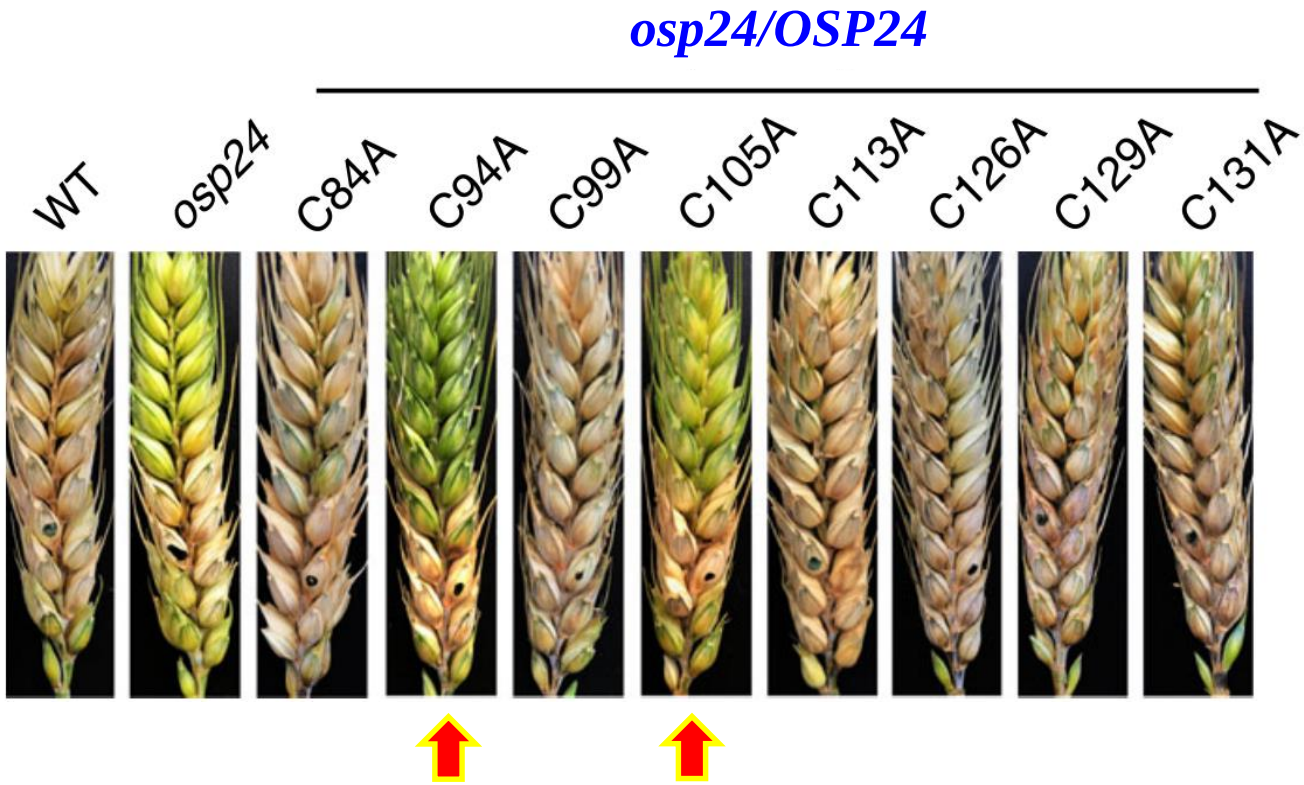
Cytoplasmic vs. apoplastic effectors in *M. oryzae*
Zhang and Xu, 2014. PLoS Pathogens

C94 and C105 are important for the function of Osp24



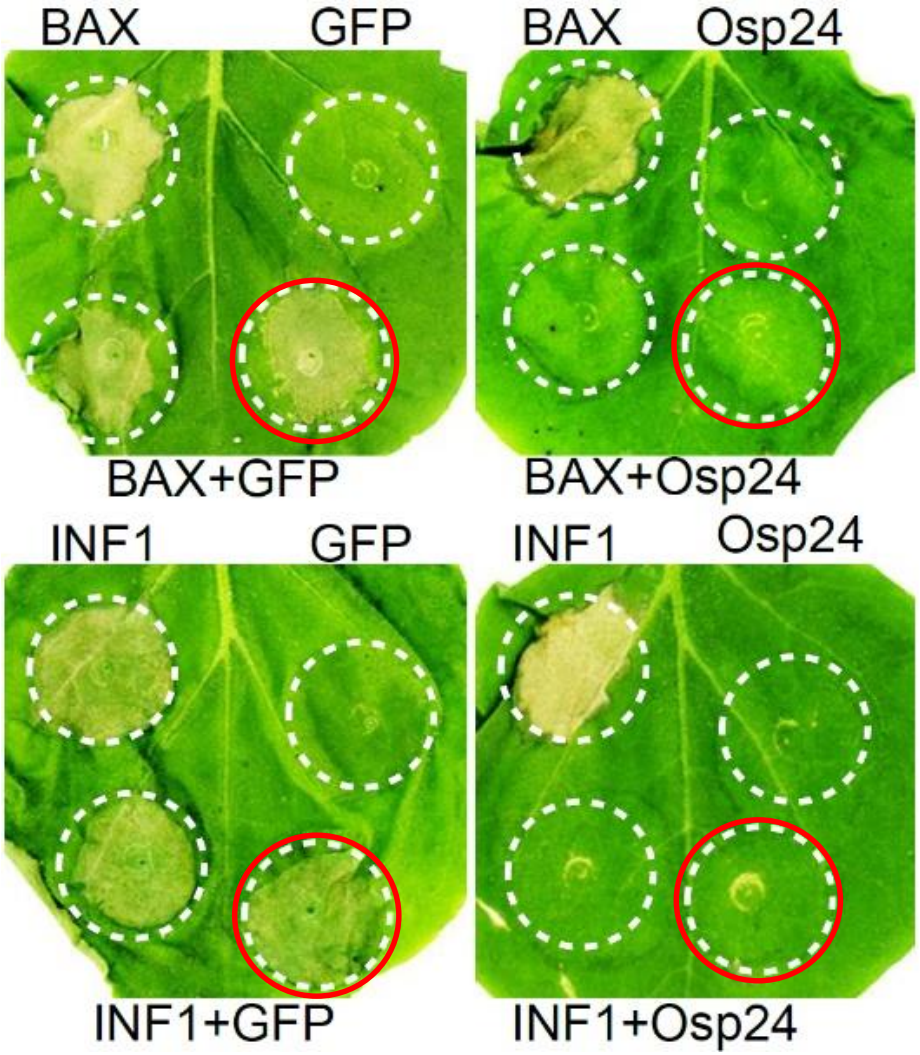
-Eight cysteine residues – alanine scan mutagenesis

-C94A and C105A mutations failed to complement *osp24*



C94A & C105A mutations affect Osp24 stability

Osp24 suppresses programmed cell death (PCD) induced by BAX or INF1



Transient expression in *Nicotiana benthamiana*

Screening for Osp24-interacting proteins

Yeast two-hybrid library –wheat heads inoculated with *F. graminearum*

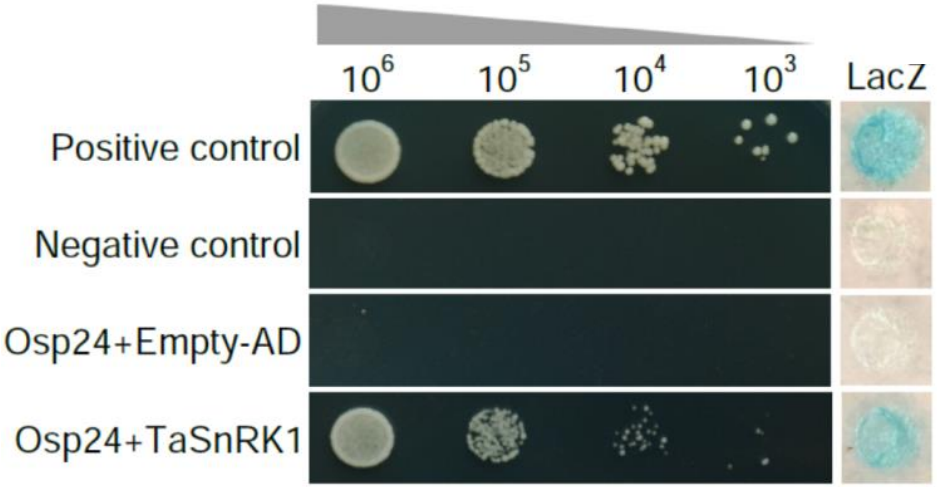
Name	Clonies	Annotation
OIC1	4,6,11,18,23	SNF1-related protein kinase 1 **(TaSnRK1)**
OIC2	2,15,19,20	SGT1 (suppressor of the G2 allele of skp1)
OIC3	1	S-acyltransferase 23
OIC4	8	Peroxisome biogensis protein 5
OIC5	14	Ribosomal protein L7
OIC6	5,30	Phosphoglycolate phosphatase
OIC7	25	Blue copper-binding protein
OIC8	12	Pre-mRNA-splicing factor SLU7

16 Osp24-interacting clones (OIC)

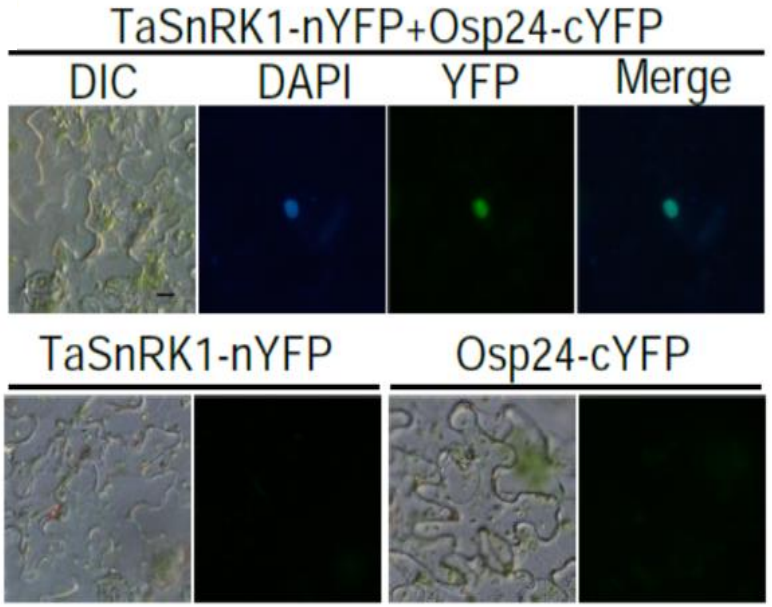
Jiang et al., 2020. Nature Communications

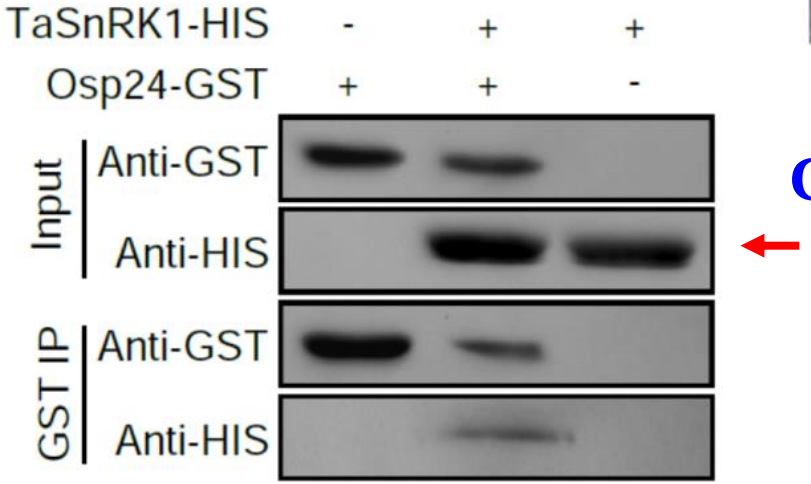
Osp24 interacts with wheat TaSnRK1

Y2H



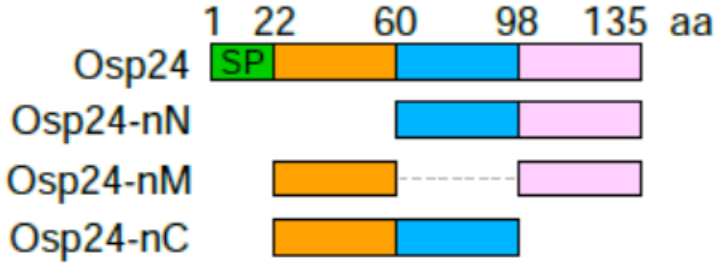
BiFC

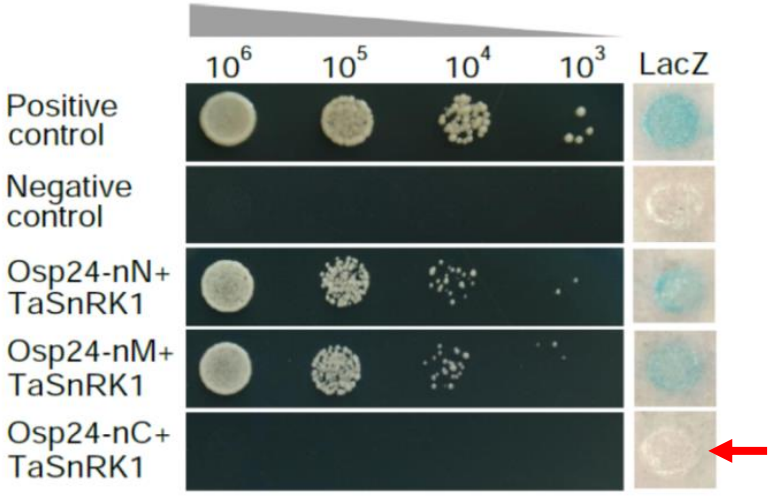




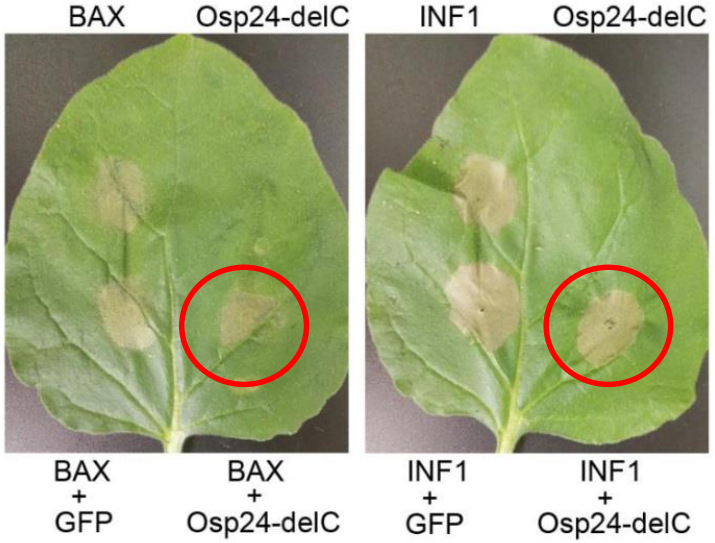
GST pull-down

The C-terminal region of Osp24 important for its interaction with TaSnRK1 and PCD suppression





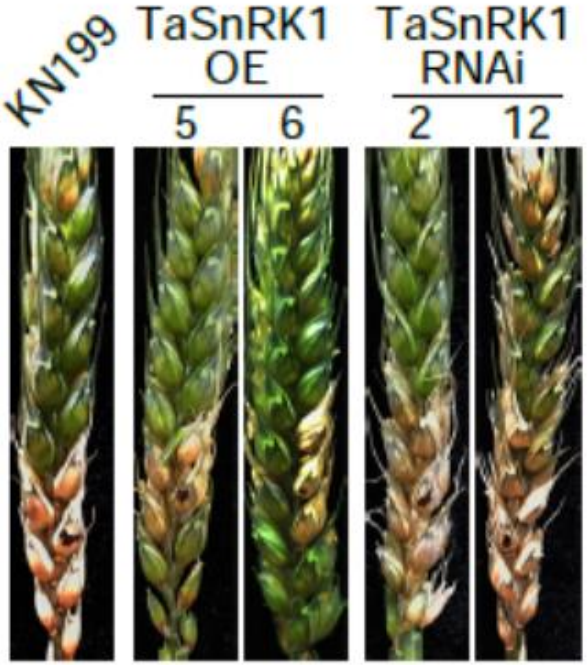
Interaction with TaSnRK1

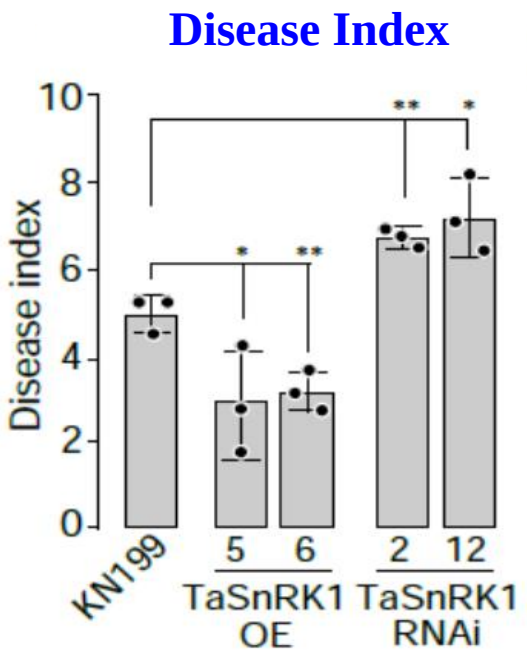


Essential for PCD suppression

TaSnRK1 contributes to FHB resistance

- Overexpressing TaSnRK1 - increased in resistance
- Silencing TaSnRK1 - decreased in resistance

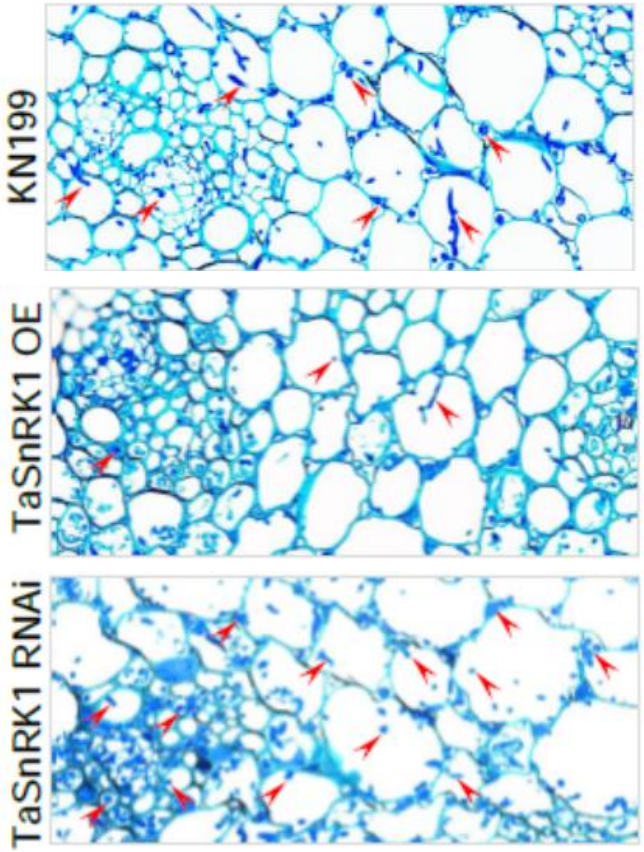




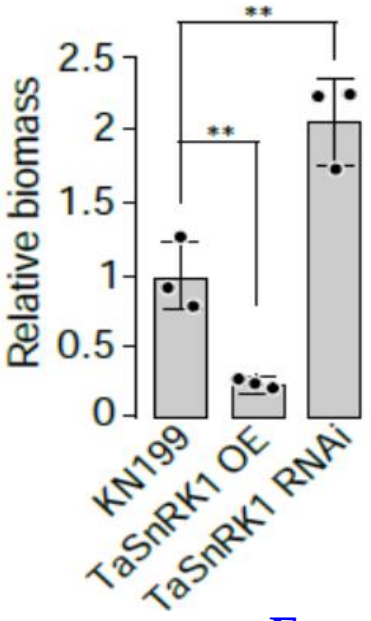
TaSnRK1 contributes to FHB resistance

Infectious growth

- reduced in TaSnRK1 OE
- increased in TaSnRK1 silencing



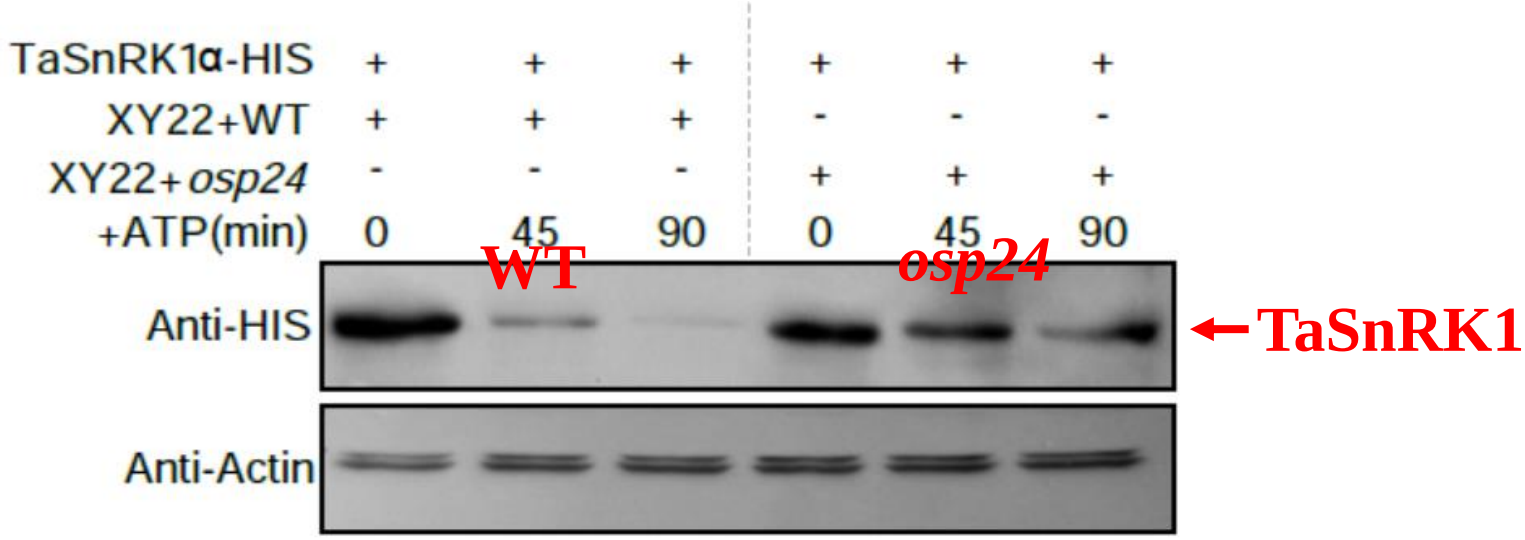
Invasive Hyphae



Fungal Biomass

Osp24 accelerates the degradation of TaSnRK1

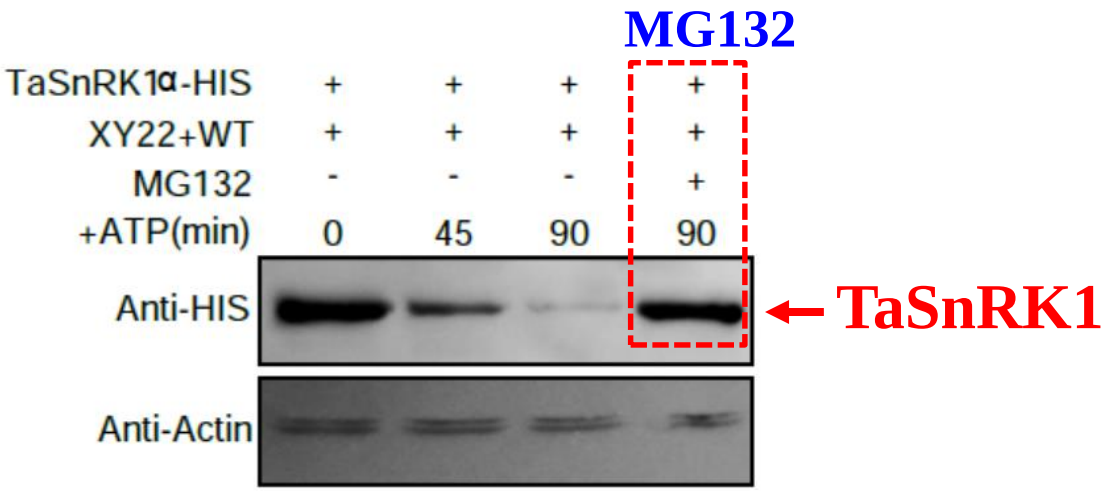
TaSnRK1 recombinant proteins co-incubated with total proteins from wheat heads inoculated with PH-1 (WT) or *osp24* mutant



In vitro degradation assays

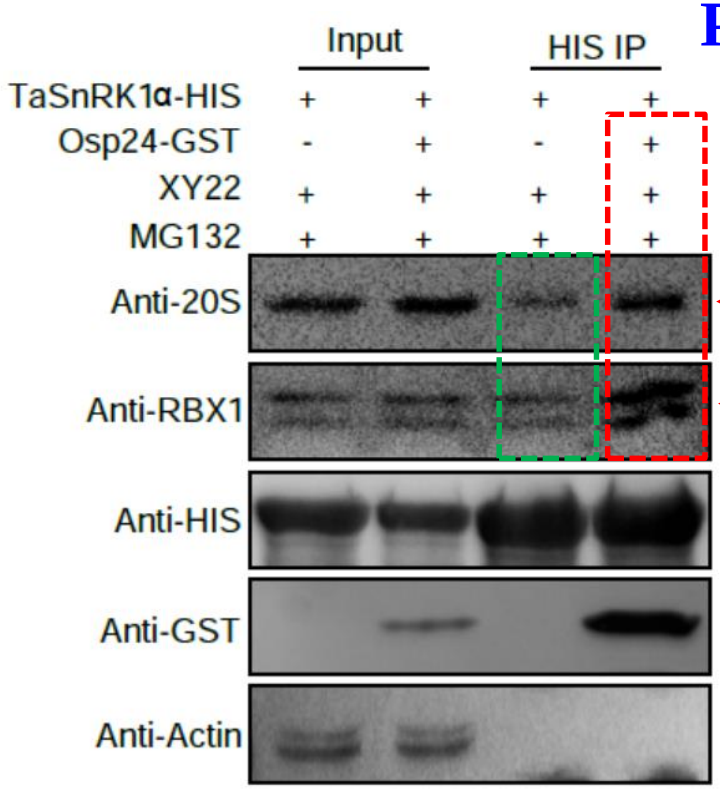
– adapted from studies with SnRK1 in Arabidopsis

Degradation of TaSnRK1 via the 26S proteasome



TaSnRK1 degradation suppressed by MG132
- an inhibitor of the 26S proteasome

Osp24 facilitates the interaction of TaSnRK1 with the SCF ubiquitin ligase and 26S proteasome

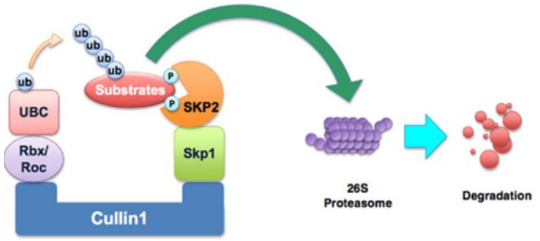


Proteins coIPed with TaSnRK1

Osp24

← Anti-20S proteasome (26S proteasome)

← Anti-RBX1 (SCF ubiquitin ligase complex)

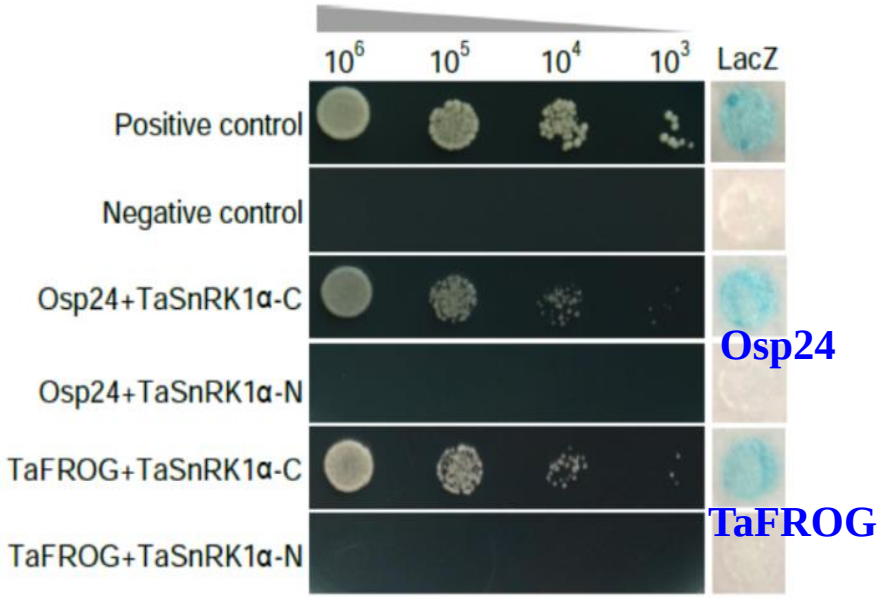


Ubiquitin-Proteasome System (UPS)

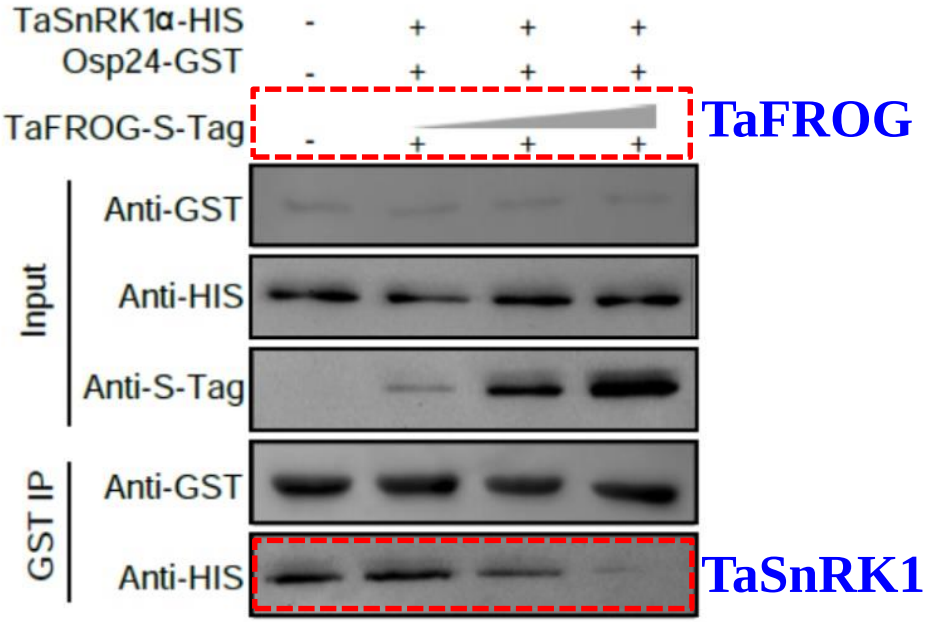
TaFROG competes with Osp24 in binding with TaSnRK1

- Transcription of wheat orphan TaFROG induced by DON
- TaSnRK1 interacts with TaFROG

Perochon et al, 2015

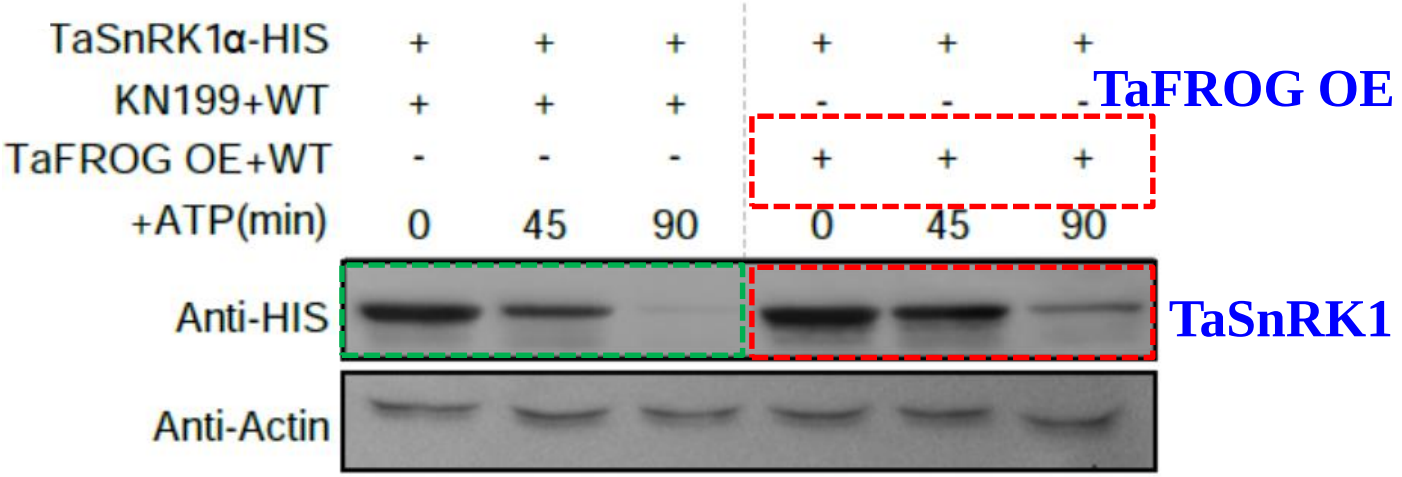


Both interact with the C-terminal region of TaSnRK1



In vitro pull down assays

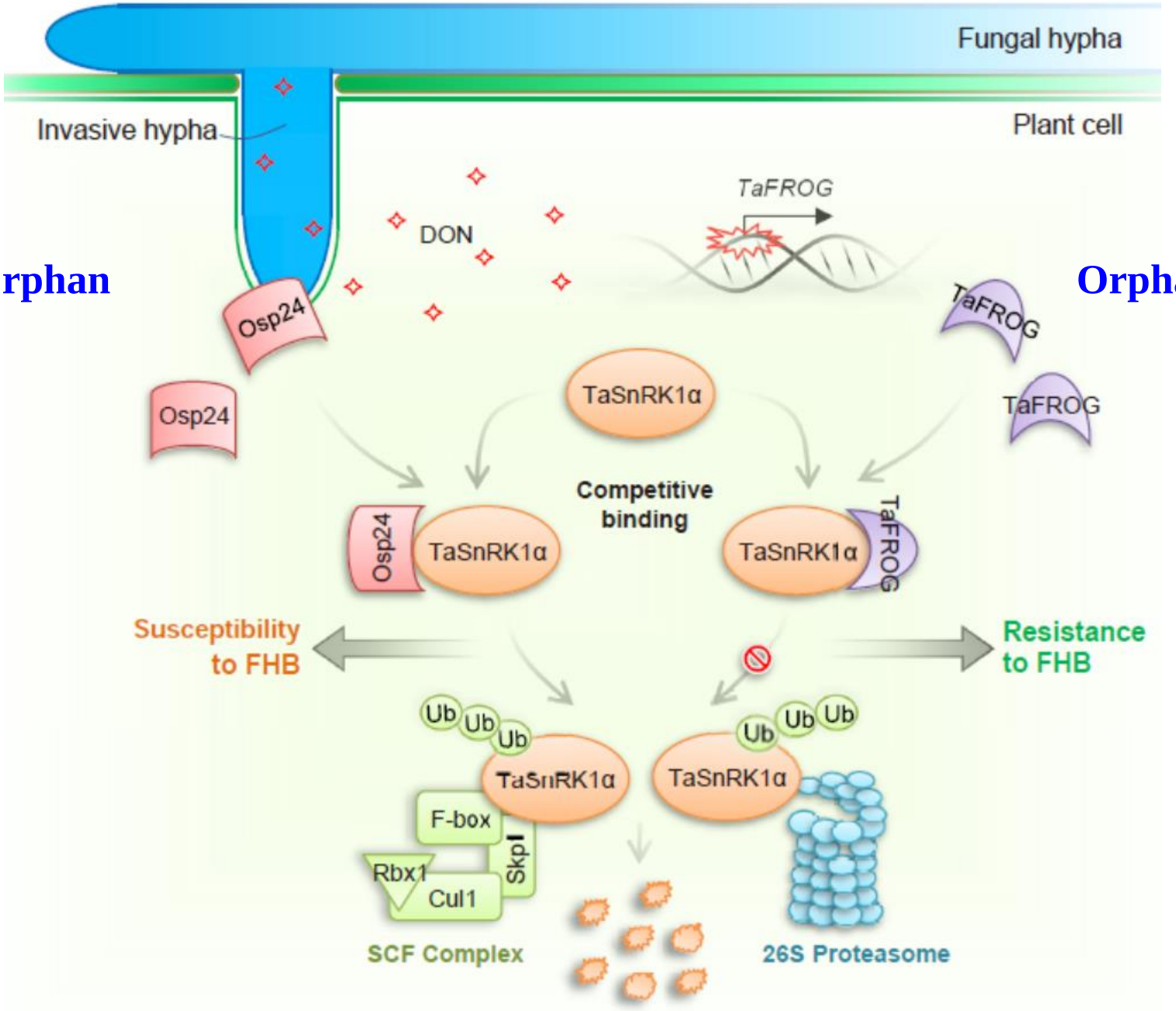
TaFROG overexpression enhances WHB resistance by stabilizing TaSnRK1



In vitro degradation assays

Orphan

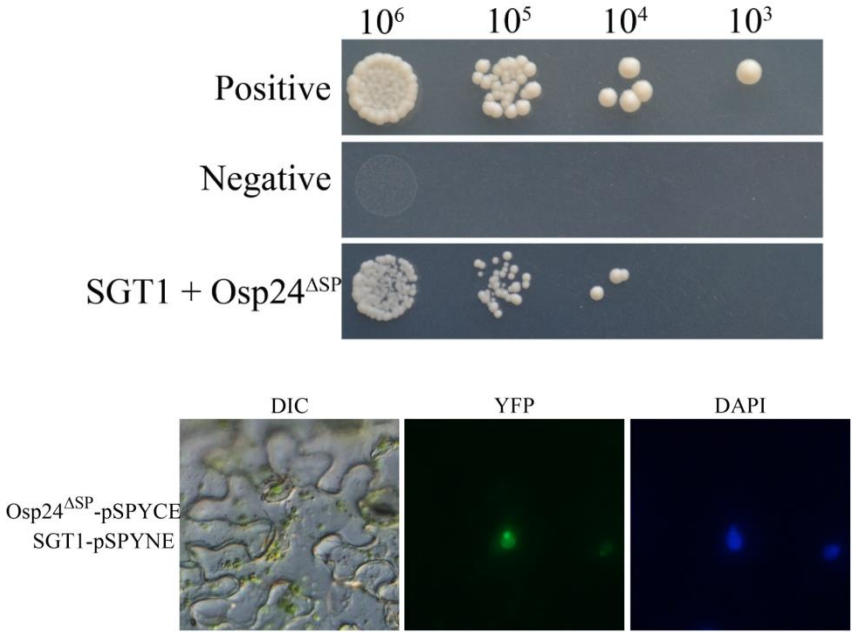
Orphan



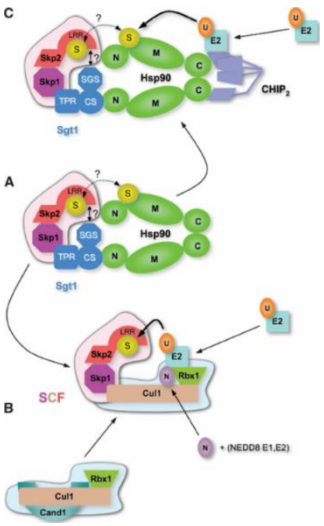
The Osp24-TaSgt1 interaction - Recruiting UPS to TaSnRK1

the ubiquitin-proteasome system (UPS)

- Osp24 also interacts with TaSgt1
- Sgt1 is associated with the SCF ubiquitin ligase complex



(Jiang et al., unpublished)



Zhang *et al.*, 2008. EMBO J.

Osp25

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Thanks for your attention!