Functional analysis of transcription factors in the cereal head blight fungus, *Fusarium graminearum*

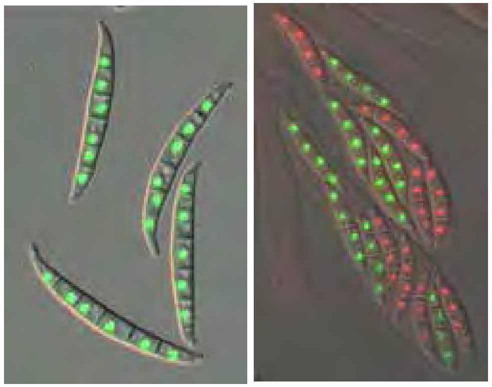
Yin-Won Lee

Center for Fungal Pathogenesis

Seoul National University

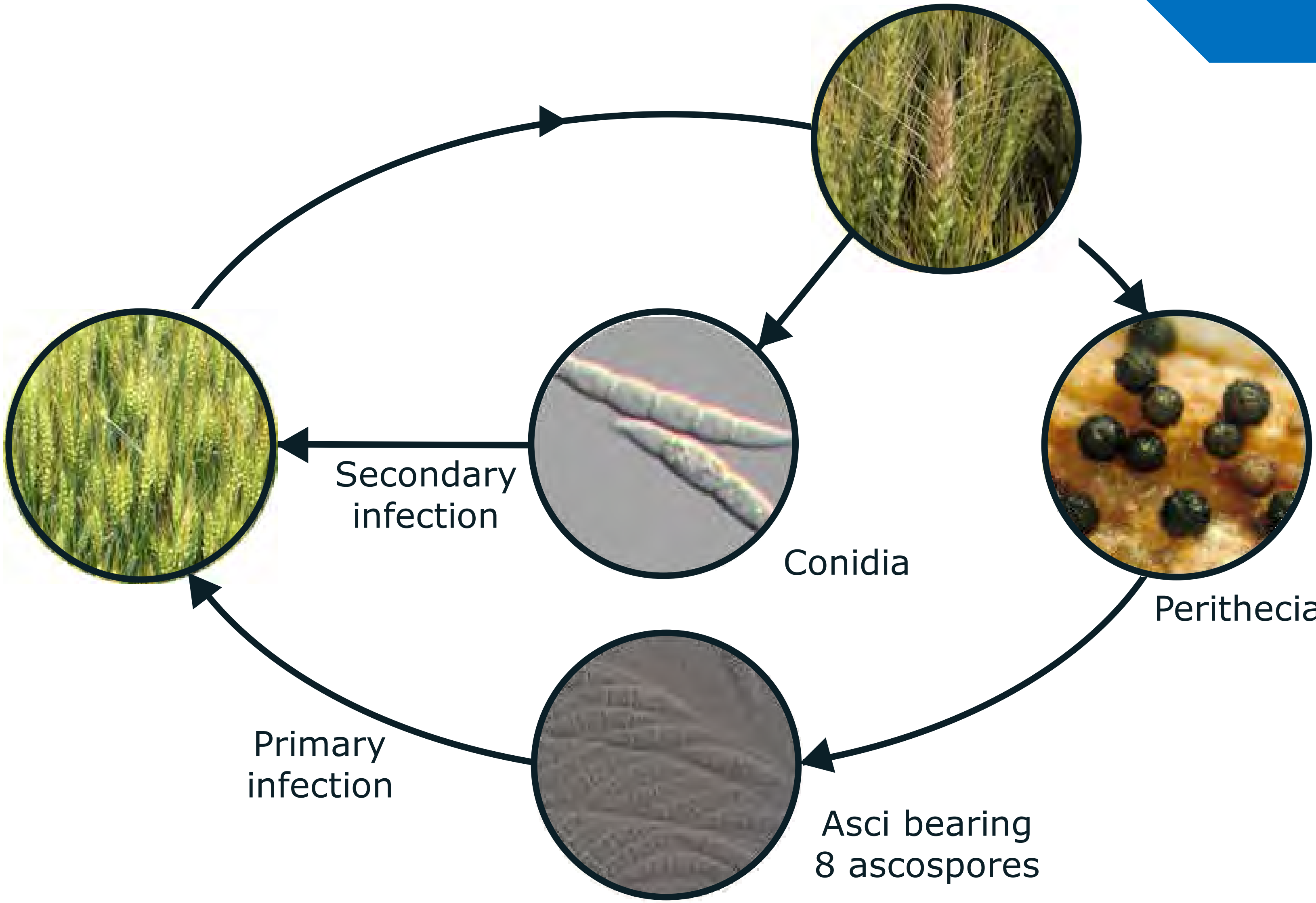
Fusarium graminearum

- Teleomorph: *Gibberella zeae*
- Ascomycete, homothallic (self-fertile)
- Causal agent of Fusarium head blight of cereal crops
- Mycotoxin producer: trichothecenes, zearalenone
- 13,332 protein-coding genes (Cuomo et al., 2007. Science)



(http://www3.abe.iastate.edu/)

Disease cycle of *F. graminearum*



Secondary infection

Conidia

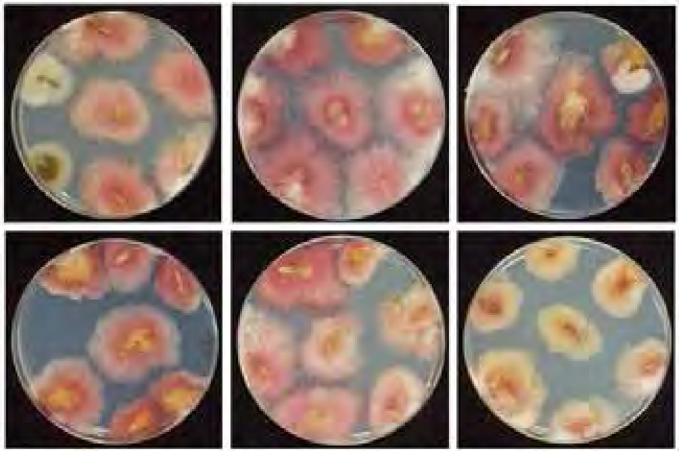
Perithecia

Primary infection

Asci bearing 8 ascospores

Head blight disease in South Korea (Wheat)

Head blight disease in South Korea (Barley)



Previous studies – **Genetic** approaches

- **Forward genetics**
  - ü 35,000 transformants were generated by Restriction Enzyme-Mediated Integration (REMI). (Kim et al., 2005, 2006, 2008)
  - ü Characterization of auxotrophic genes (Han et al., 2004; Kim et al., 2007; Seo et al., 2007)
  - ü Chitin synthases (Kim et al., 2009), F-box protein (Han et al., 2007), syntaxins (Hong et al., 2010)
  - ü Ascospore discharge (Min et al., 2010)
  - ü Acetyl coenzyme A synthesis (Lee et al., 2011; Son et al., 2011, 2012a, 2012b)

- **Reverse genetics**
  - ü G-protein signaling components (Yu et al., 2008)
  - ü Sucrose non-fermenting protein kinase 1 (Lee et al., 2009)
  - ü Isocitrate lyase (Lee et al., 2009)
  - ü Carotenoid biosynthetic pathway (Jin et al., 2010)
  - ü Meiotic silencing (Son et al., 2011)
  - ü Component of velvet complex (Lee et al., 2012)
  - ü Regulators of G-protein signaling (Park et al., 2012)
  - ü Function of peroxisomes (Min et al., 2012)

Genome-wide **TF deletion** project

- Transcription factors (TFs) orchestrate gene expression under the control of cellular signaling pathways and are key mediators of cellular function.

- TFs are involved in the biological processes including virulence and toxin production.

- Only 13 TFs were characterized in *F. graminearum*.

- About 90 pathogenesis-related genes have been characterized.

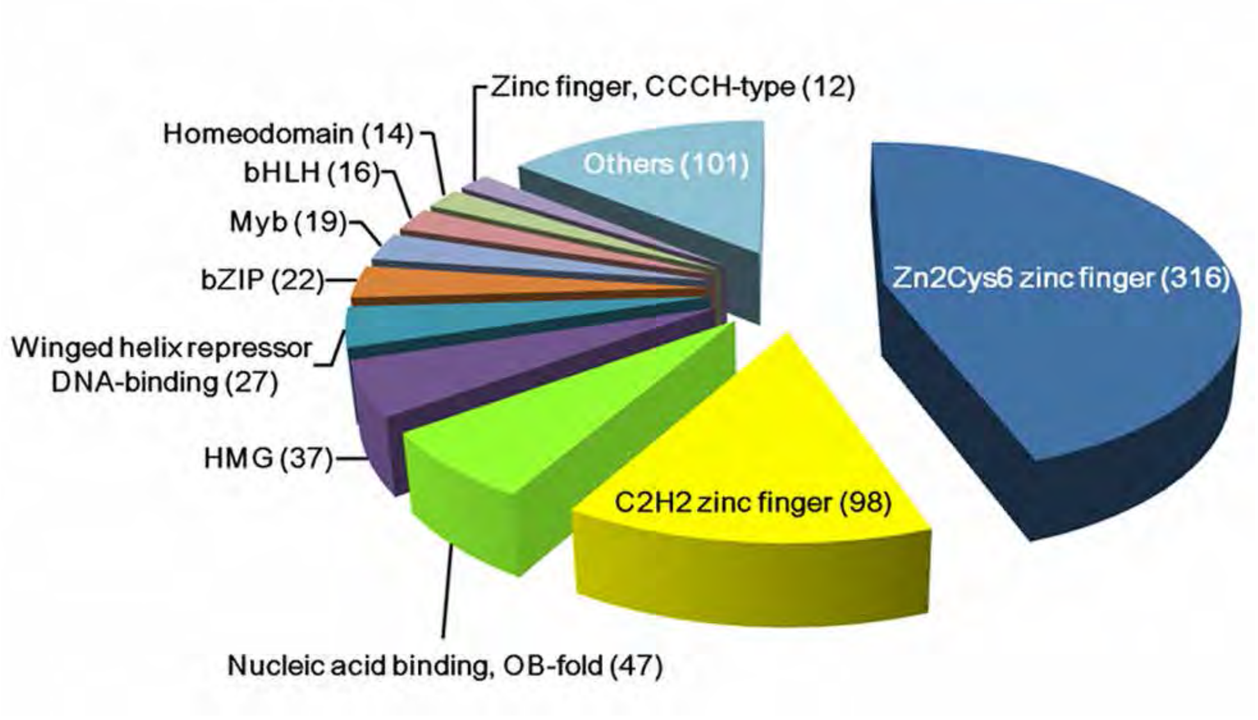
⇨ **Genome-wide TF deletion project**

TFs in *F. graminearum*

FTFD Fungal Transcription Factor Database

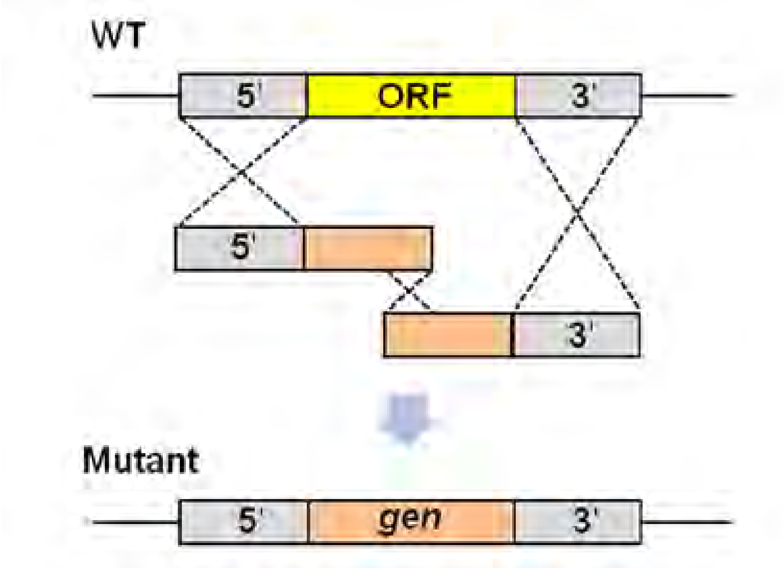
(Park et al., 2008. Bioinformatics)

- 693 TFs were selected from 45 sub-family.
- 16 TFs were manually selected.

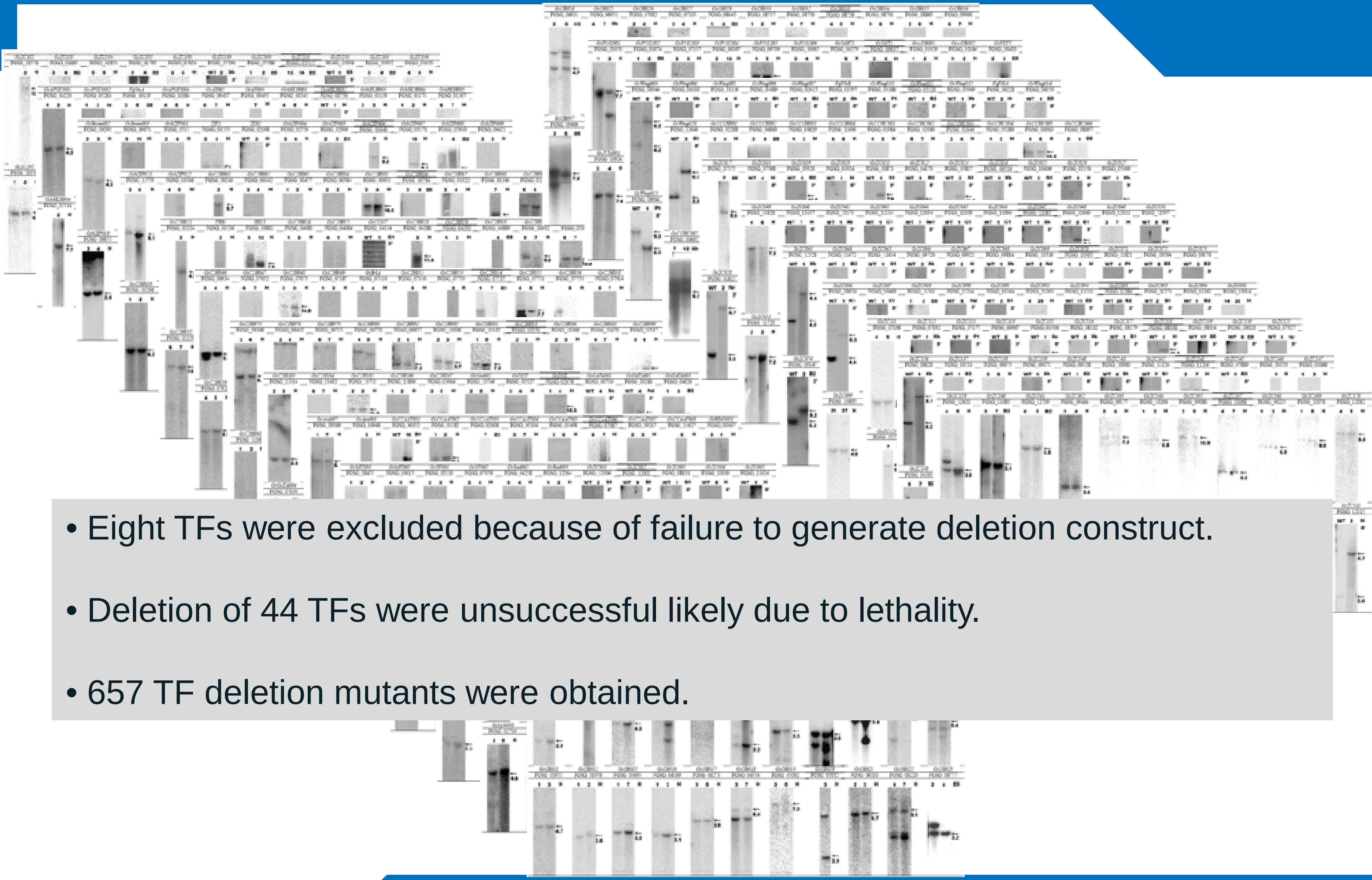


Deletion strategy
– Triple homologous recombination using **split marker**

Triple homologous recombination



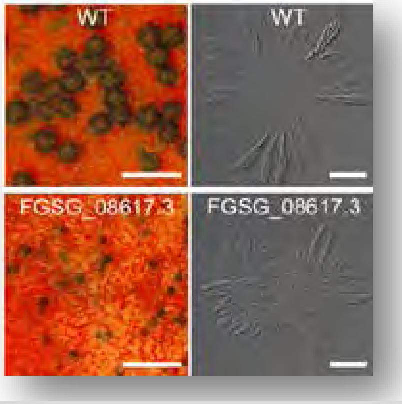
Deletion confirmation by Southern blot

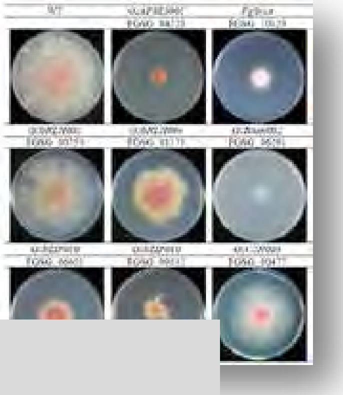


- Eight TFs were excluded because of failure to generate deletion construct.
- Deletion of 44 TFs were unsuccessful likely due to lethality.
- 657 TF deletion mutants were obtained.

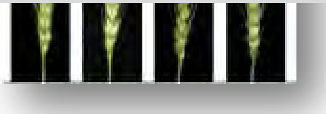
Phenotyping

- Vegetative growth (0 ~ 5)
- Sexual development (0 ~ 5)
- Conidia production (0 ~ 5)



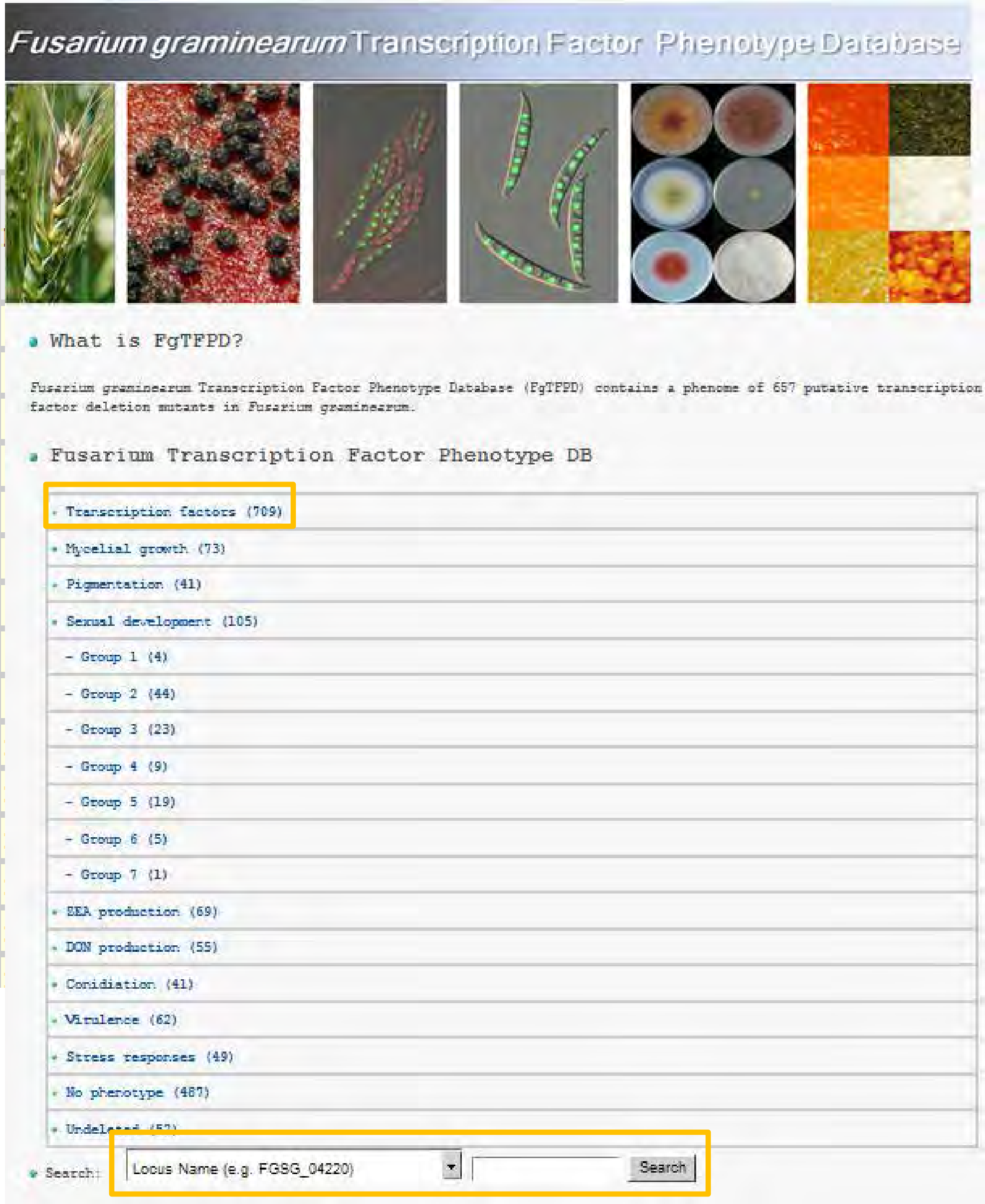


- 11,000 phenotypes (phenome) were obtained from
- 17 phenotype categories.



- Stress responses (0 ~ 4)

Fusarium graminearum Transcription Factor Phenotype Database



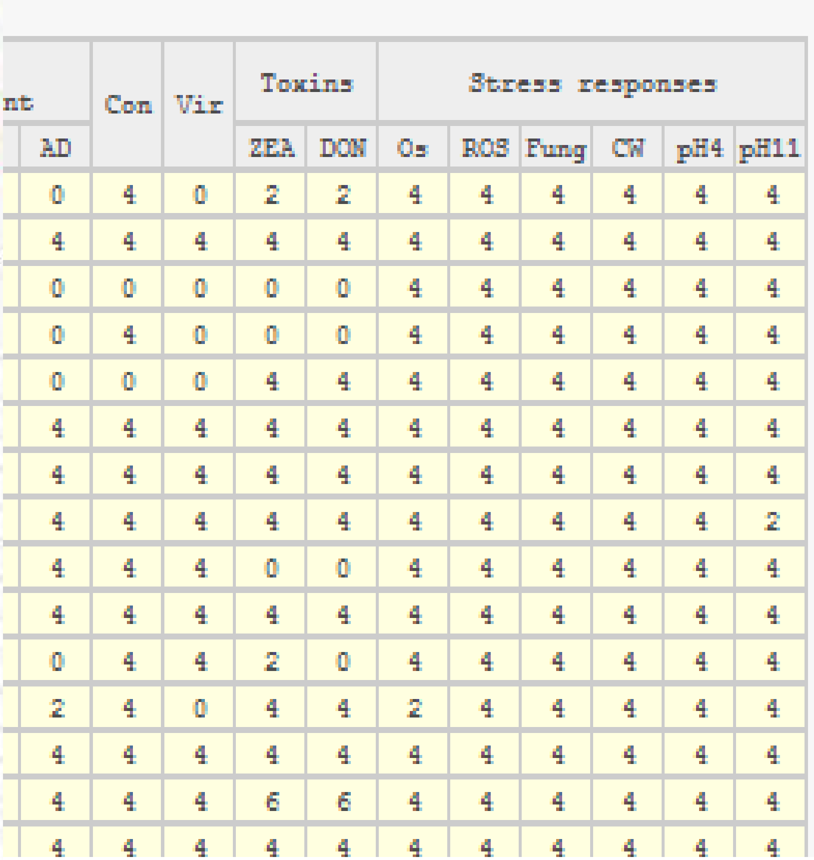
Fusarium graminearum Transcription Factor Phenotype Database

- What is FgTFPD?

Fusarium graminearum Transcription Factor Phenotype Database (FgTFPD) contains a phenome of 657 putative transcription factor deletion mutants in Fusarium graminearum.

- Fusarium Transcription Factor Phenotype DB

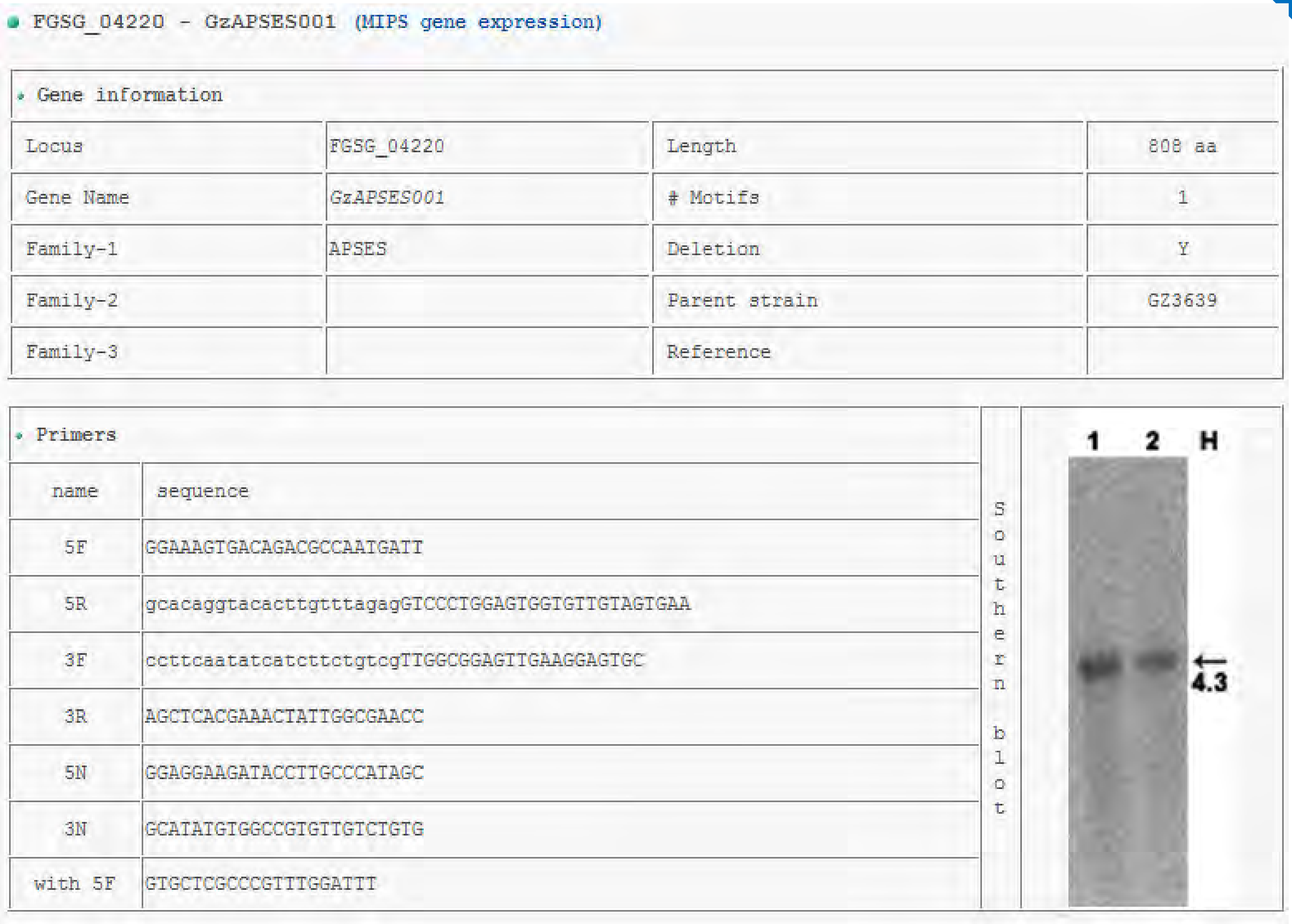
- Transcription factors (709)
- Mycelial growth (73)
- Pigmentation (41)
- Sexual development (105)
  - Group 1 (4)
  - Group 2 (44)
  - Group 3 (23)
  - Group 4 (9)
  - Group 5 (19)
  - Group 6 (5)
  - Group 7 (1)
- ZEA production (69)
- DON production (55)
- Conidiation (41)
- Virulence (62)
- Stress responses (49)
- No phenotype (487)
- Undeleted (57)



nt	Con	Vir	Toxins		Stress responses					
AD			ZEA	DON	Os	ROS	Fung	CW	pH4	pH11
0	4	0	2	2	4	4	4	4	4	4
4	4	4	4	4	4	4	4	4	4	4
0	0	0	0	0	4	4	4	4	4	4
0	4	0	0	0	4	4	4	4	4	4
0	0	0	4	4	4	4	4	4	4	4
4	4	4	4	4	4	4	4	4	4	4
4	4	4	4	4	4	4	4	4	4	4
4	4	4	4	4	4	4	4	4	4	2
4	4	4	0	0	4	4	4	4	4	4
4	4	4	4	4	4	4	4	4	4	4
0	4	4	2	0	4	4	4	4	4	4
2	4	0	4	4	2	4	4	4	4	4
4	4	4	4	4	4	4	4	4	4	4
4	4	4	6	6	4	4	4	4	4	4
4	4	4	4	4	4	4	4	4	4	4

(http://ftfd.snu.ac.kr/FgTFPD)

Fusarium graminearum Transcription Factor Phenotype Database



FGSG_04220 - GzAPSES001 (MIPS gene expression)

Gene information

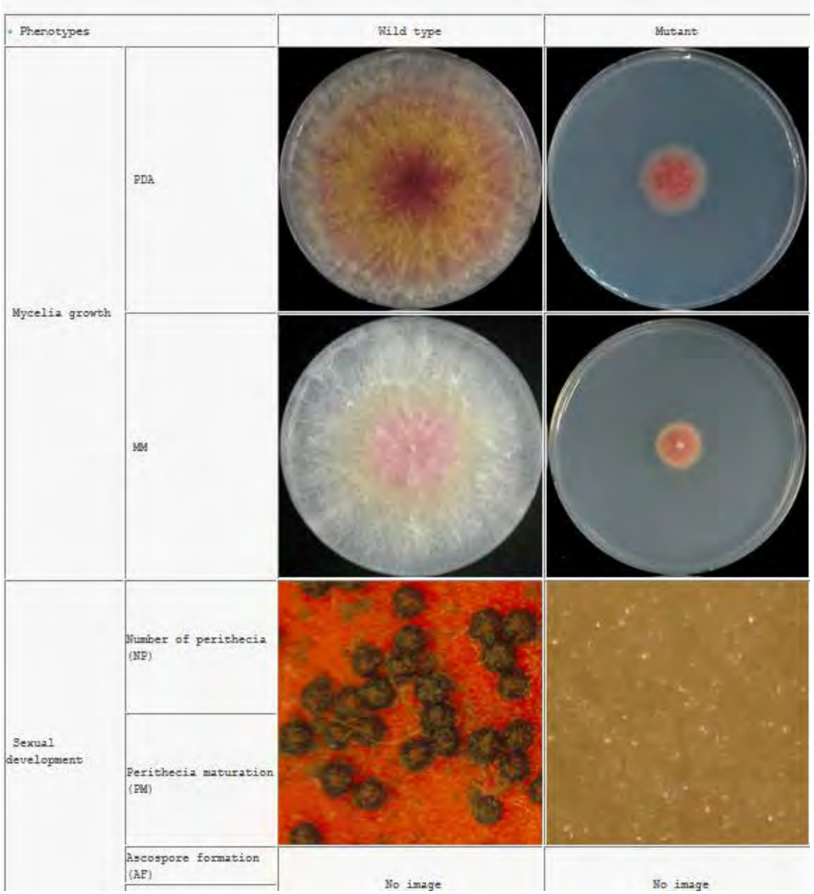
Locus	FGSG_04220	Length	808 aa
Gene Name	*GzAPSES001*	# Motifs	1
Family-1	APSES	Deletion	Y
Family-2		Parent strain	GZ3639
Family-3		Reference	

Primers

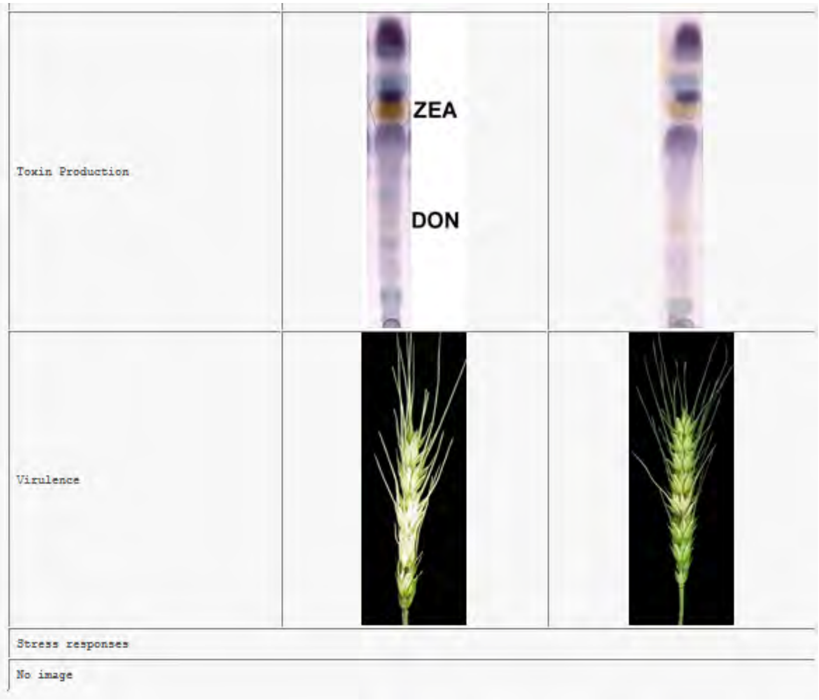
name	sequence
5F	GGAAAGTGACAGACGCCAATGATT
5R	gcacaggtacacttgtttagagGTCCCTGGAGTGGTGTTGTAGTGAA
3F	ccttcaatatcatcttctgtcgTTGGCGGAGTTGAAGGAGTGC
3R	AGCTCACGAAACTATTGGCGAACC
5N	GGAGGAAGATACCTTGCCCATAGC
3N	GCATATGTGGCCGTGTTGTCTGTG
with 5F	GTGCTCGCCCGTTTGGATTT

Southern blot

Fusarium graminearum Transcription Factor Phenotype Database

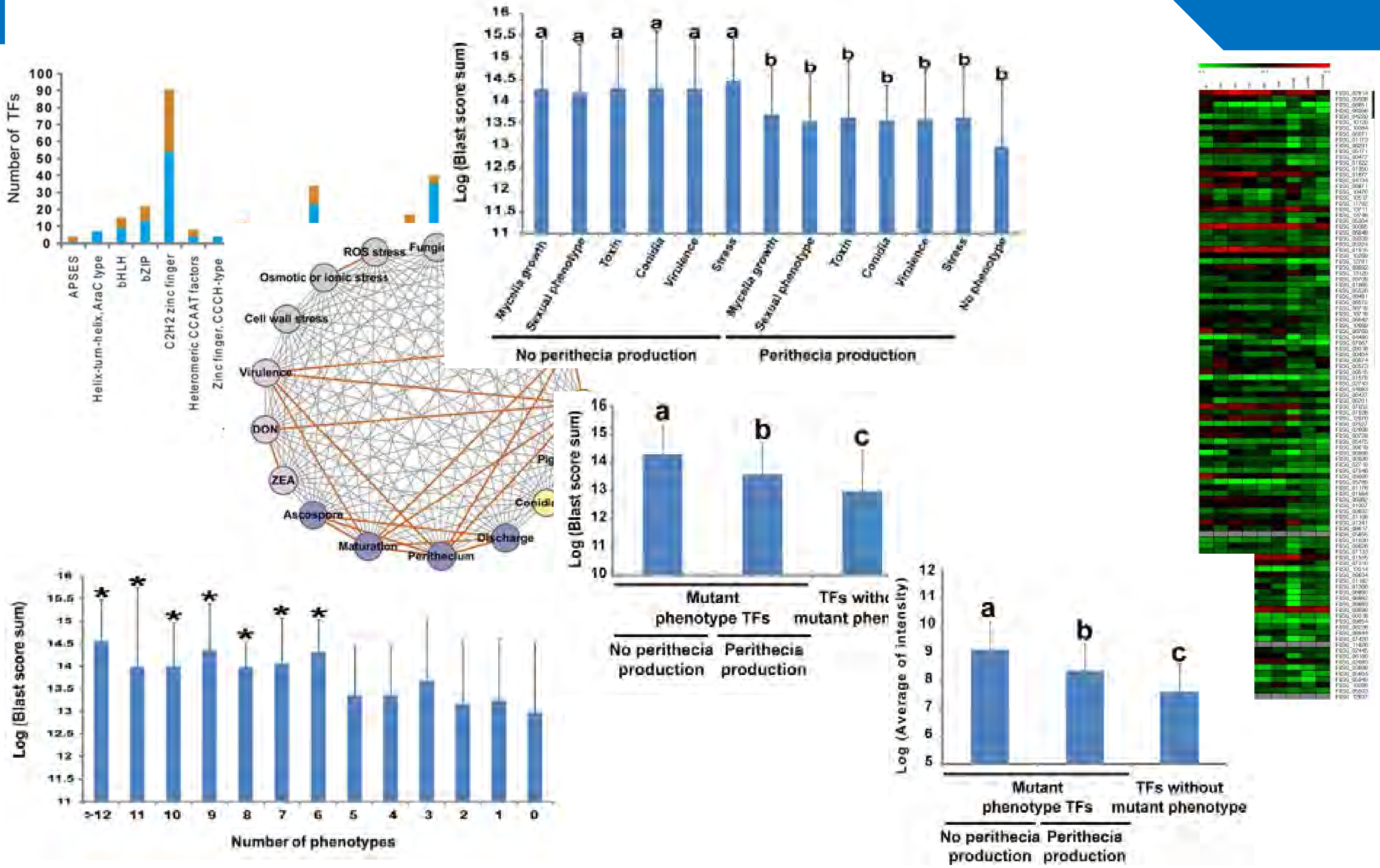


Phenotypes		Wild type	Mutant
Mycelia growth	PDA		
	MM		
Sexual development	Number of perithecia (NP)		
	Perithecia maturation (PM)		
	Ascospore formation (AF)	No image	No image



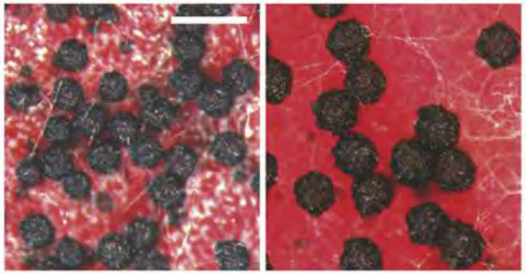
Toxin Production		
Virulence		
Stress responses		
No image		

Phenome analyses

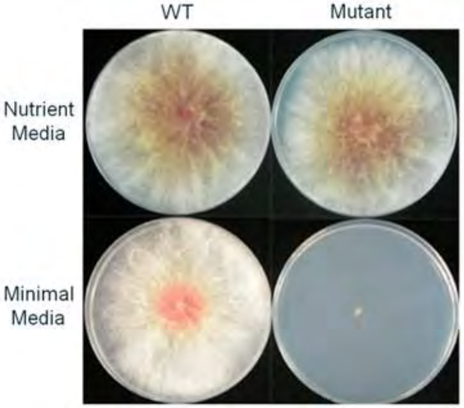


In-depth functional analysis

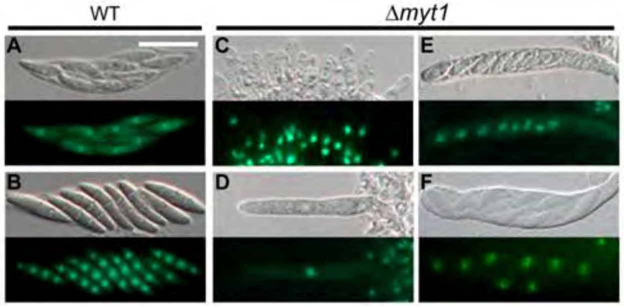
- Female fertility (Lin et al., 2011. PLoS One)
- Perithecium size (Lin et al., 2012. PLoS One)
- Nitrogen metabolism (Min et al., 2012. FEMS Microbiol. Lett.)
- Sexual development (Kim et al., Unpublished; Lee et al., Unpublished)
- Virulence (Lee et al., Unpublished, Kim et al., Unpublished)
- Zearalenone production (Park et al., Unpublished)
- Trichothecene production (Kim et al., Unpublished)
- Conidium production (Son et al., Unpublished; Min et al., Unpublished; Lee et al., Unpublished)



(Lin et al., 2012. PLoS One)



(Min et al., 2012. FEMS Microbiol. Lett.)

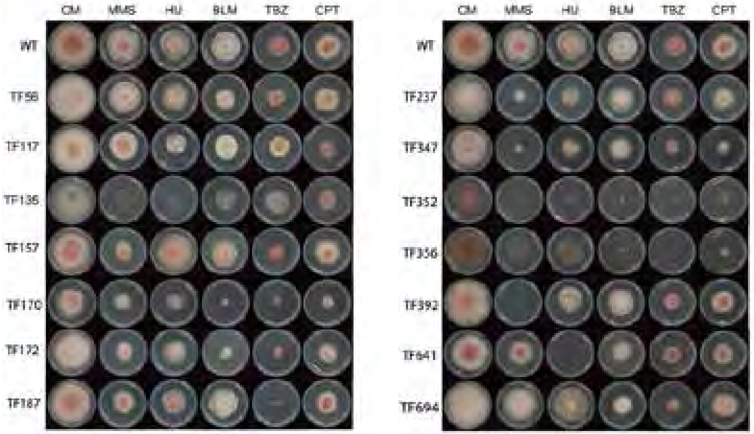


(Lin et al., 2011. PLoS One)

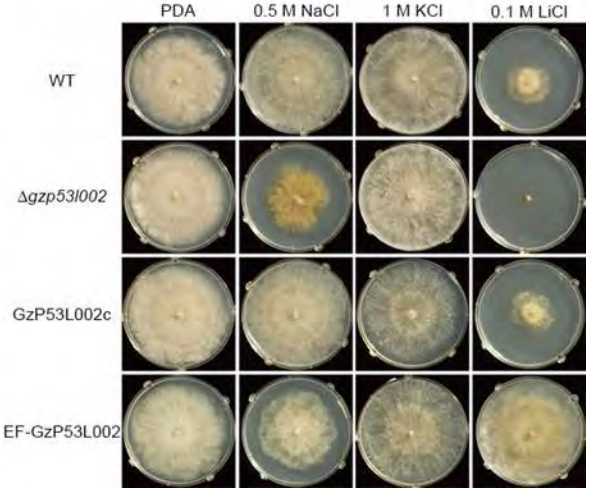
Screening mutant library

- Microcycle conidiation

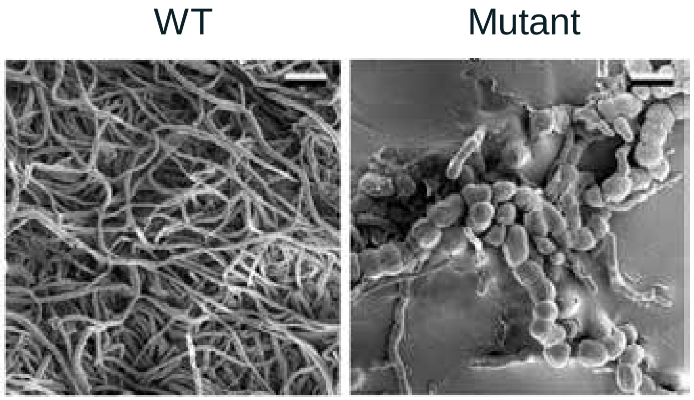
- DNA damage, cell cycle



- Sodium tolerance

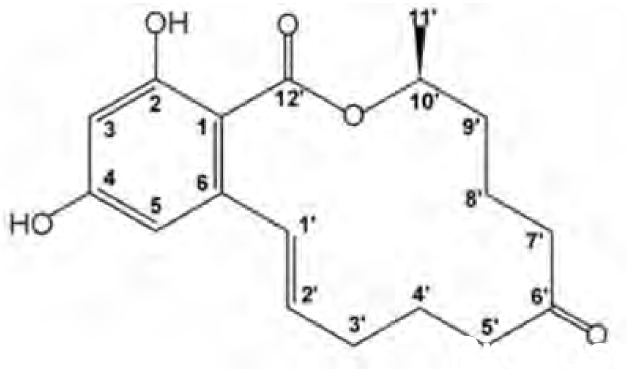


- Chlamydospore production



ZEB2 transcription factor required for zearalenone biosynthesis

Zearalenone (F-2 Toxin)

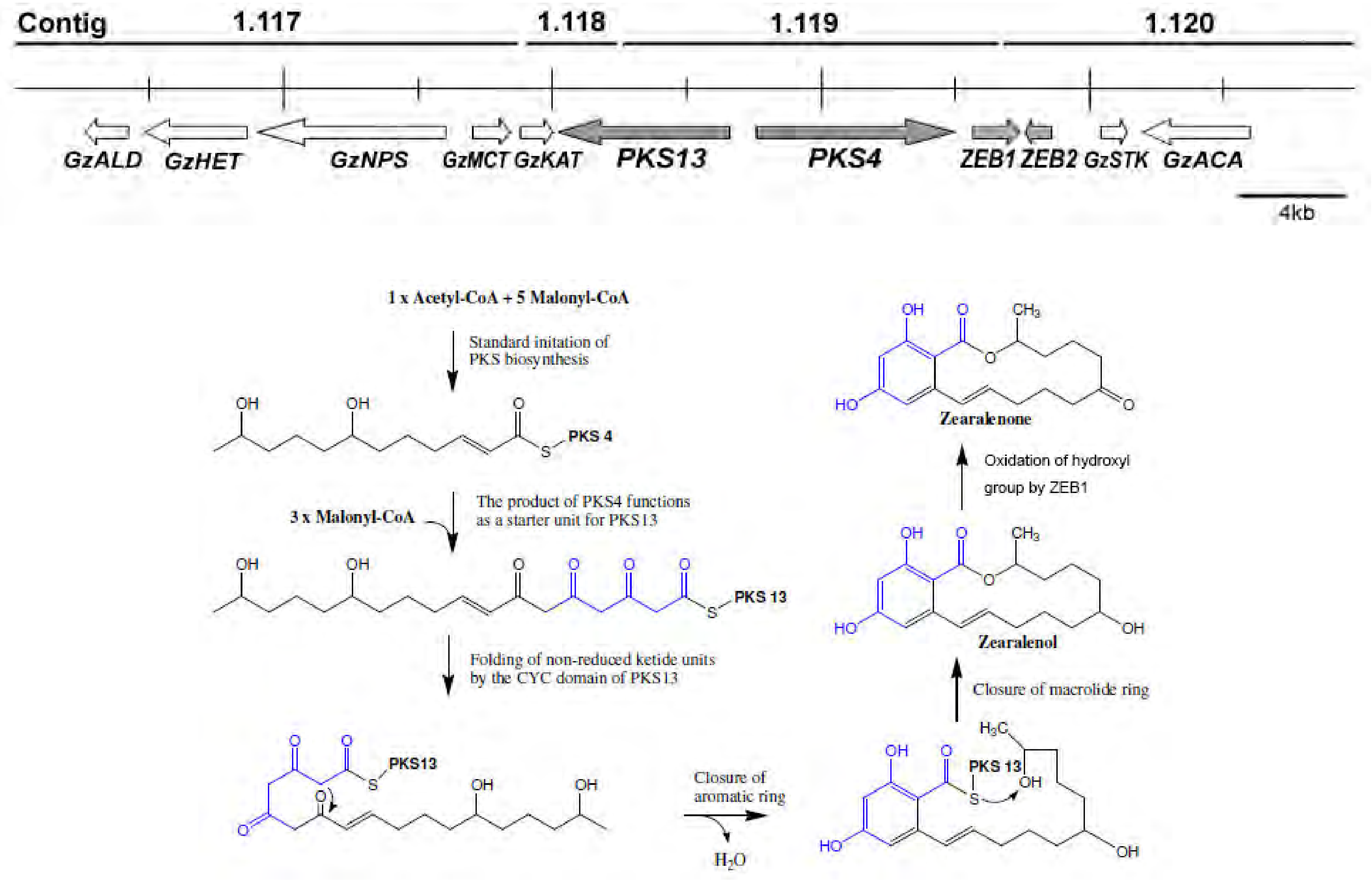


Structure

Hyperestrogenic syndrome

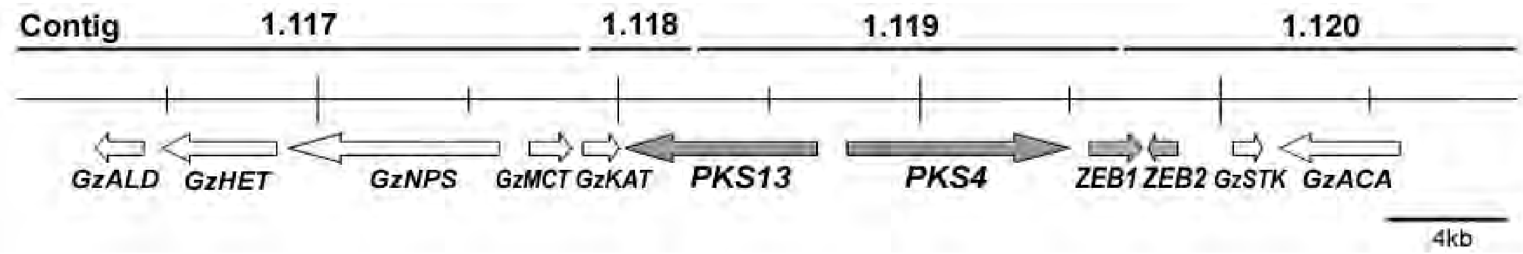
- Polyketide secondary metabolite
- Produced mainly by *F. graminearum* and *F. culmorum*
- Estrogenic syndrome
- The primary toxin causing infertility, abortion or other breeding problems in swine

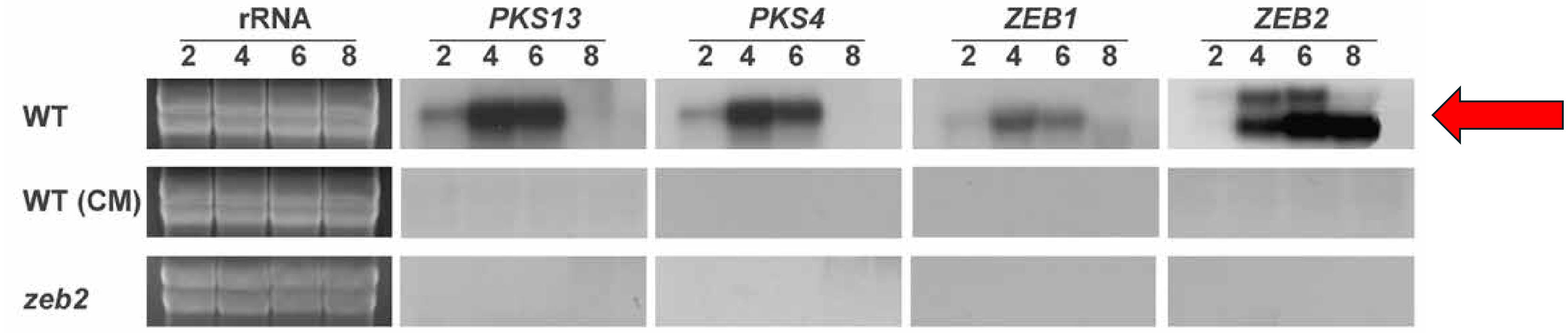
Zearalenone biosynthetic pathway



(Kim et al., 2005, Mol. Microbiol.)

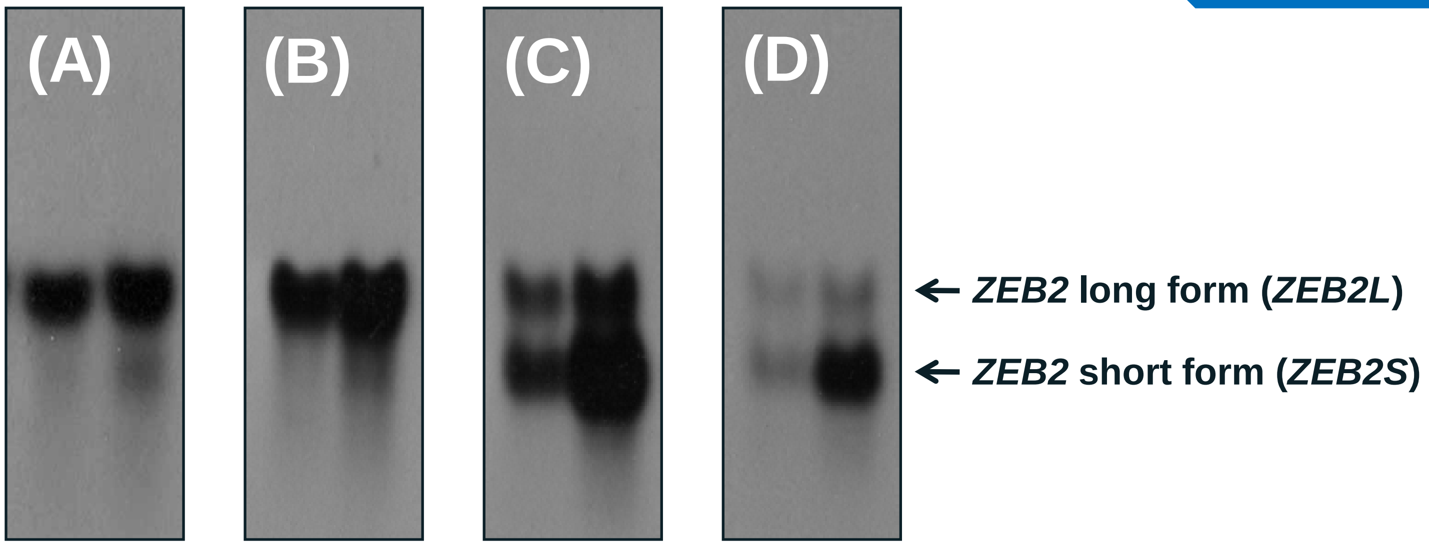
bZIP transcriptional regulator, ZEB2





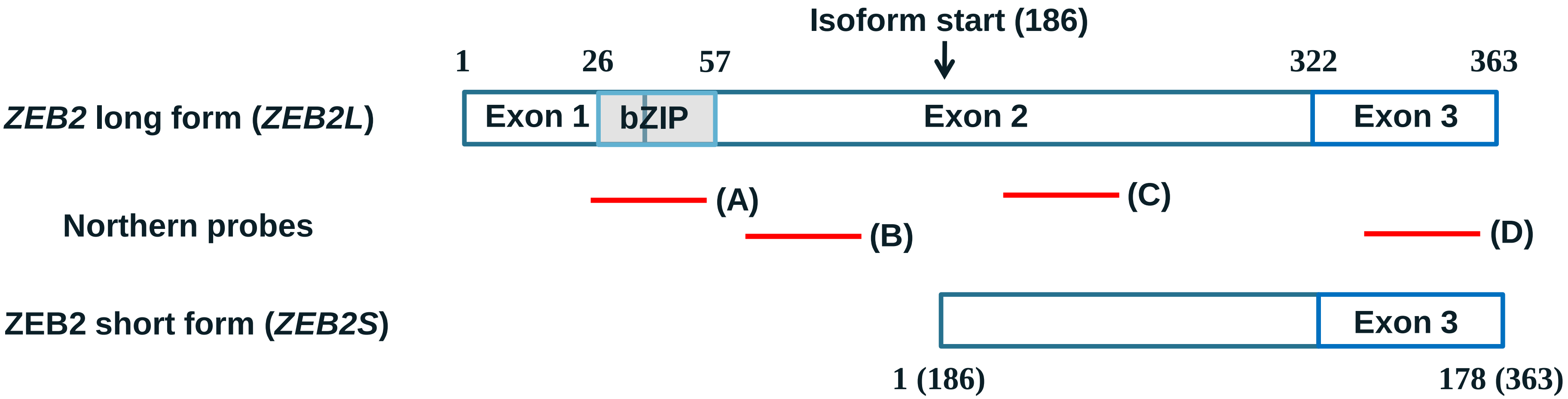
(Kim et al., 2005, Mol. Microbiol.)

Identification of *ZEB2* transcripts



← *ZEB2* long form (*ZEB2L*)

← *ZEB2* short form (*ZEB2S*)



Isoform start (186)

1 26 57 322 363

ZEB2 long form (*ZEB2L*)

Exon 1 bZIP Exon 2 Exon 3

Northern probes

(A) (B) (C) (D)

ZEB2 short form (*ZEB2S*)

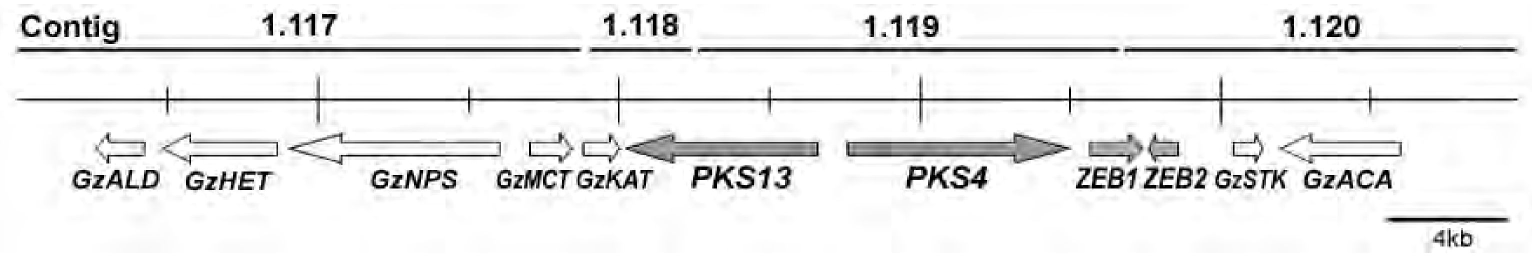
Exon 3

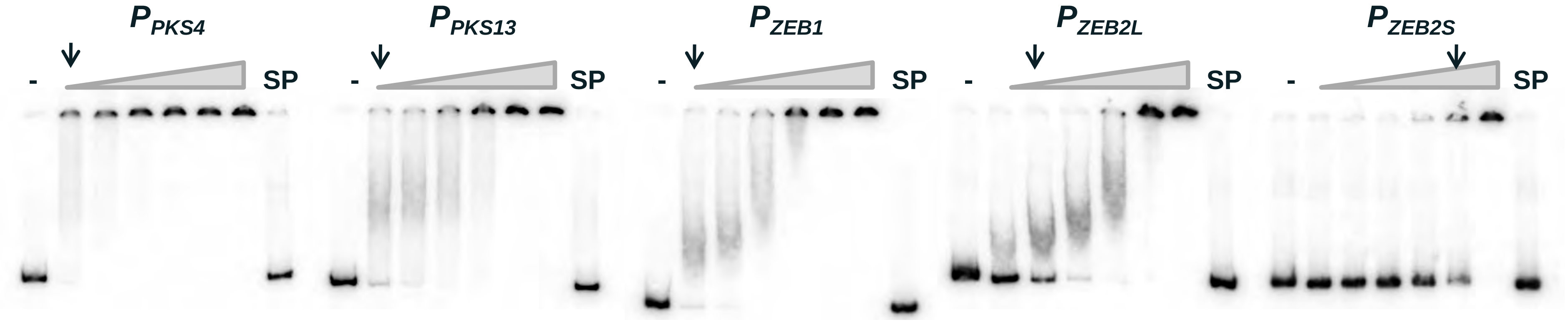
1 (186) 178 (363)

Identification of *ZEB2* transcripts

```
1    aacggccgtgaccttaattcagcttaaATGACATTGGTTGACAATATGCAATCTACCGAGTCGTTCCATGCGTTACCCACTCGGTCTGAT
1                                M  T  L  V  D  N  M  Q  S  T  E  S  F  H  A  L  P  T  R  S  D
91   GTTGAGGACCCCAACGAGAGACGCAAGATTCAAAACAGGATTGCTCAAAAGAAACATAgtgtgacattccaccgctcgcacatgtatttc
22    V  E  D  P  N  E  R  R  K  I  Q  N  R  I  A  Q  K  K  H
181  aactaatcagtctccagGACAAAAGATGAAGCGGCGAATTGAAGAGCTTGAGACCAAAGTCAACAACCAATGCCAAACGTCGAATTGGAC
41                    R  Q  K  M  K  R  R  I  E  E  L  E  T  K  V  N  N  Q  C  Q  T  S  N  W  T
271  CAGCCACGCACCCACAGATTCCTGCCAAGAGCAGCAATTCCTTGACAATACAGACTTTGGACTGATGCTAGAGGATGATTTGCTATACCG
66     S  H  A  P  T  D  S  C  Q  E  Q  Q  F  L  D  N  T  D  F  G  L  M  L  E  D  D  L  L  Y  R
361  TGAACTTTCCGCCAGCCTCGACGGTGCGGGATTAACCGCTGTGGCCCAAATGCATGATTCTCCACGACCGAATCAGCAGCAAAGACTTTC
96     E  L  S  A  S  L  D  G  A  G  L  T  A  V  A  Q  M  H  D  S  P  R  P  N  Q  Q  Q  R  L  S
451  GGTTTCGGGCATGCCTAGCAGCCCGACCTCCACCAGCAACGTTGCACAGCGAGGCCTTTCTATCGGCGATCATAGCTCAGCTTCCAACCA
126    V  S  G  M  P  S  S  P  T  S  T  S  N  V  A  Q  R  G  L  S  I  G  D  H  S  S  A  S  N  H
541  TTTAAGCTCTTTATCTTTAGTTCCTGGATCGACAGAAGGGAGTCTTCCAACTCGACAGCACGATAATCTGTGCAACCAAGACCTCCGCGA
156    L  S  S  L  S  L  V  P  G  S  T  E  G  S  L  P  T  R  Q  H  D  N  L  C  N  Q  D  L  R  D
631  CATGGTGCCCGAGGAGAAAATGAGTCGTATTCTCAAGGTTATTCAGGATGCGGGTTACAAAGACATGGATTCATTCATGACTGAGTATTA
186    M  V  P  E  E  K  M  S  R  I  L  K  V  I  Q  D  A  G  Y  K  D  M  D  S  F  M  T  E  Y  Y
721  TGTGAGGGATTTCGATGCTTCGTCACACGTCAGCGCCGTCCAAAGGCAGAGTCGAAGTCGGCGGTTGAGAGGCTTCTTGGAGCAGCTACG
216    V  R  D  F  D  A  S  S  H  V  S  A  V  Q  R  Q  S  R  S  R  R  L  R  G  F  L  E  Q  L  R
811  AGTCGGTGCAGAAAGCTGGTCTGACTATGAAGCACATGATTACCAACAAGAGATATCTAAATCAGCCGAAGCCATCTACGCCAAAGAACT
246    V  G  A  E  S  W  S  D  Y  E  A  H  D  Y  Q  Q  E  I  S  K  S  A  E  A  I  Y  A  K  E  L
901  CGATGTCTTTAGCACCACCACTCTCGGAGAAAATGCTTCTTTCCAAGGACTGGCCAGTTTGTATCGAGTGCTGCAGAGCACCGTAGGGTC
276    D  V  F  S  T  T  T  L  G  E  N  A  S  F  Q  G  L  A  S  L  Y  R  V  L  Q  S  T  V  G  S
991  GGATATTGAGAACCATCTCCGACACGAACAGTCGATGATGCAGAGACAGgttagtgagatagttcaccgccaagtatataccttcaatcc
306    D  I  E  N  H  L  R  H  E  Q  S  M  M  Q  R  Q
1081 agctcaatgctaacaaattcgatgatacagCTTCCTCACCTCTGGTCTCTGATATCCGAGCTTTCCGGCTACCATTCGTCCGGAGAGAGA
322                                 L  P  H  L  W  S  L  I  S  E  L  S  G  Y  H  S  S  G  E  R
1171 TTGAGTTGGCGCACGGTTTCAACTGTCATTGCATTACTACAAGCCTCCCGTATGTCGCAAAATTGAtgtaactatattgatggacatggg
342   L  S  W  R  T  V  S  T  V  I  A  L  L  Q  A  S  R  M  S  Q  N  *
1261 aactagatcactagaacacagagctagaaactagaactataaagacttgcaca
```

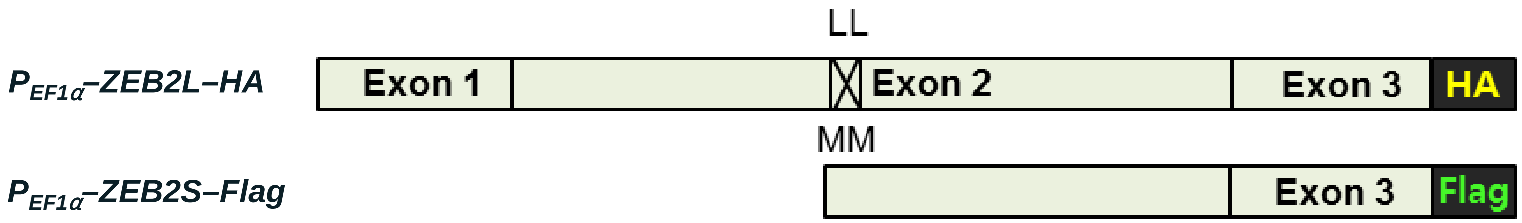
Electrophoretic Mobility Shift Assay (EMSA)

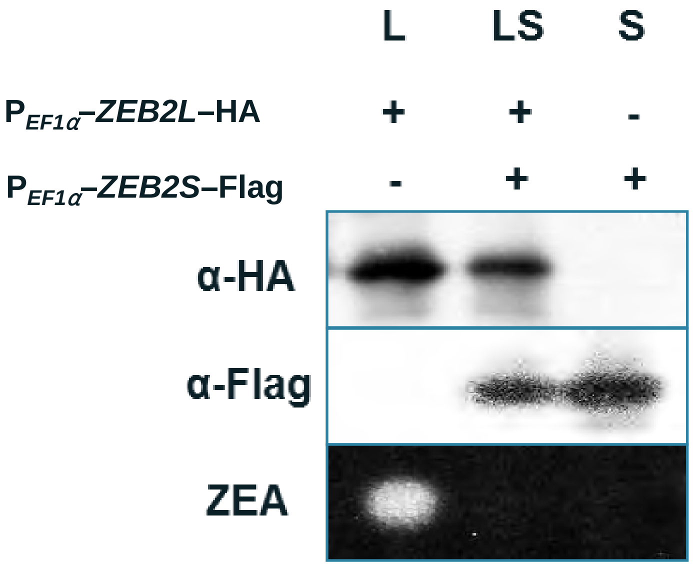




- ZEB2L proteins directly bind and activate ZEA biosynthetic genes.
- *ZEB2* gene is autoregulated by ZEB2L.
- ZEB2S proteins might have another functions for ZEA production.

Roles of two ZEB2 isoforms



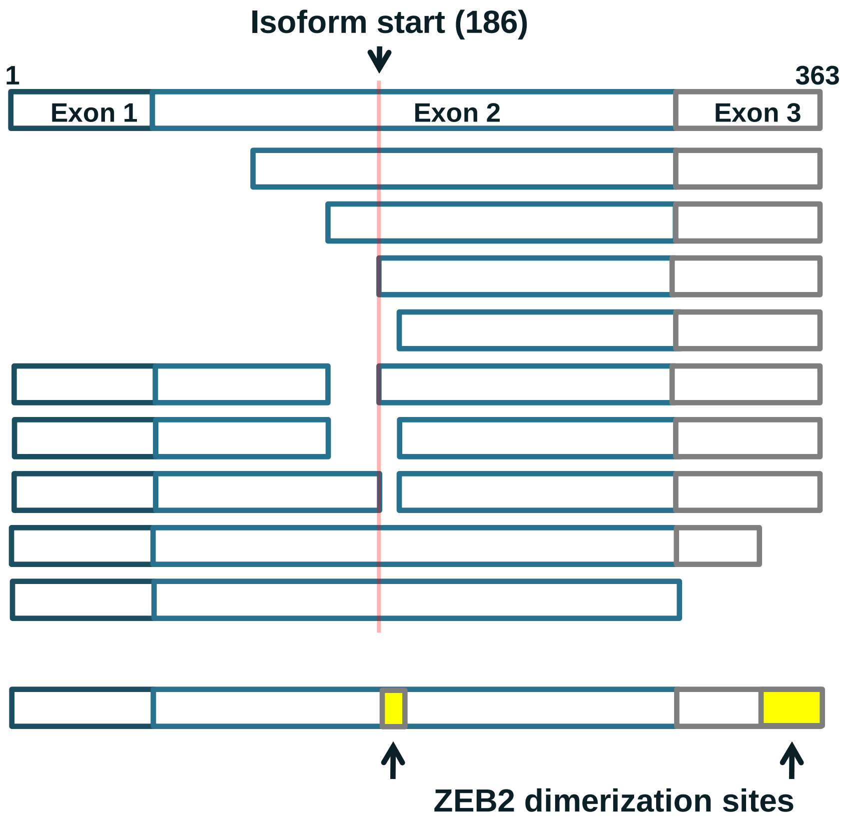


- ZEB2L alone is enough for activation of other ZEA biosynthetic genes.

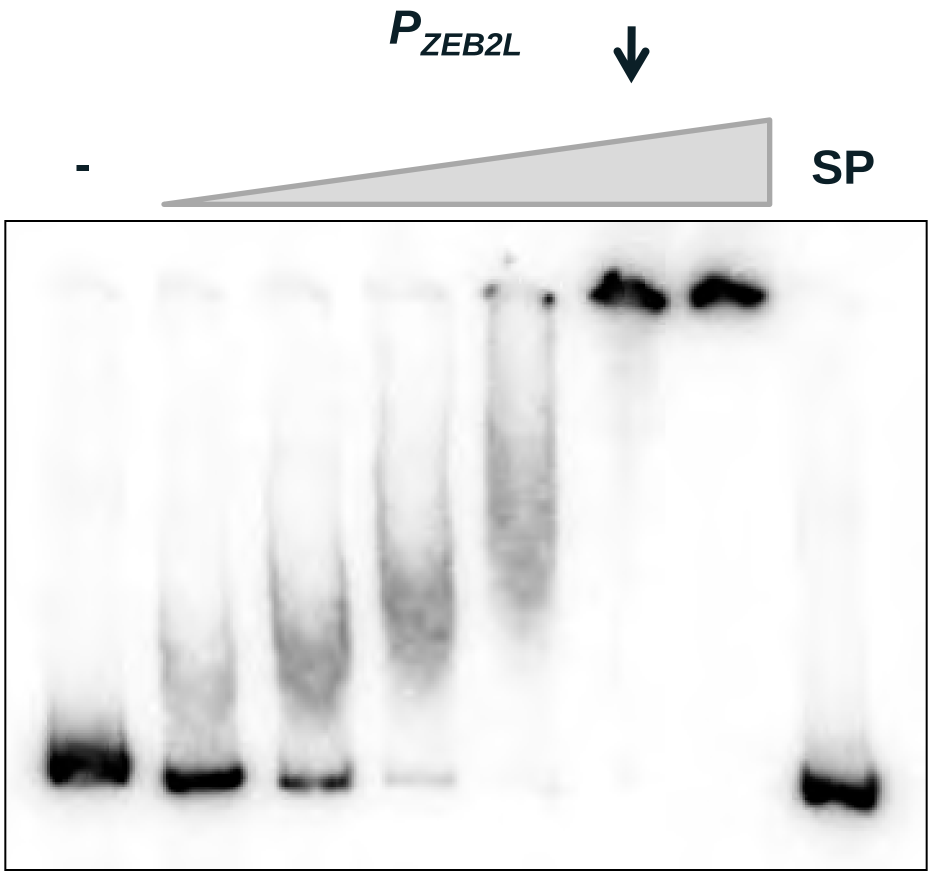
- ZEB2S functions as a repressor for ZEA production.

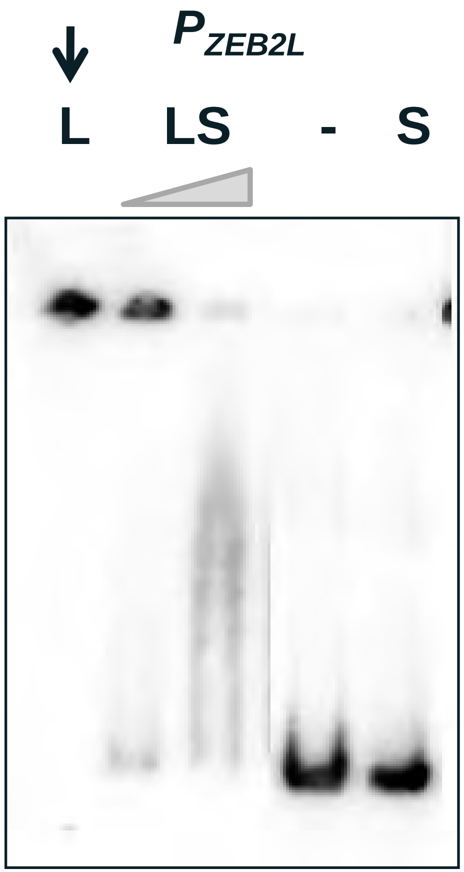
Yeast two-hybrid interactions of ZEB2L and ZEB2S

pAD	pBD	
	ZEB2L	ZEB2S
ZEB2L	+++	+++
Δ1-128	+++	++++
Δ1-172	++++	++++
Δ1-185	+++	++++
Δ1-191	+	-
Δ173-185	++	-
Δ173-191	-	-
Δ186-191	+	-
Δ338-363	-	-
Δ321-363	-	-
Empty vector	-	-



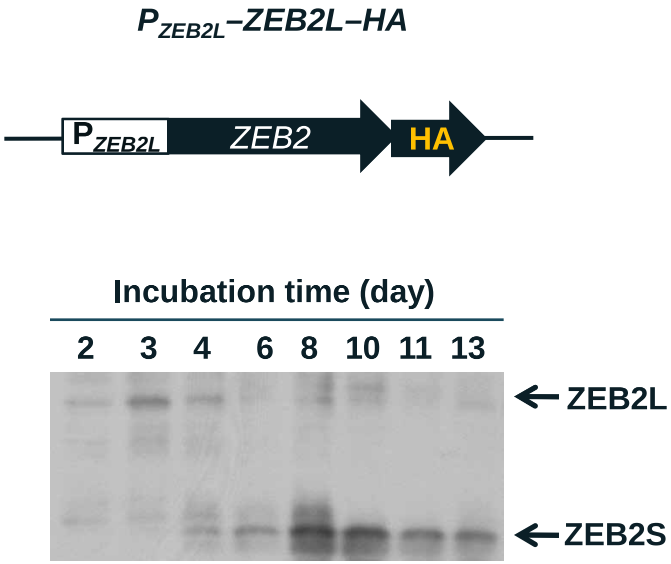
Electrophoretic Mobility Shift Assay (EMSA)

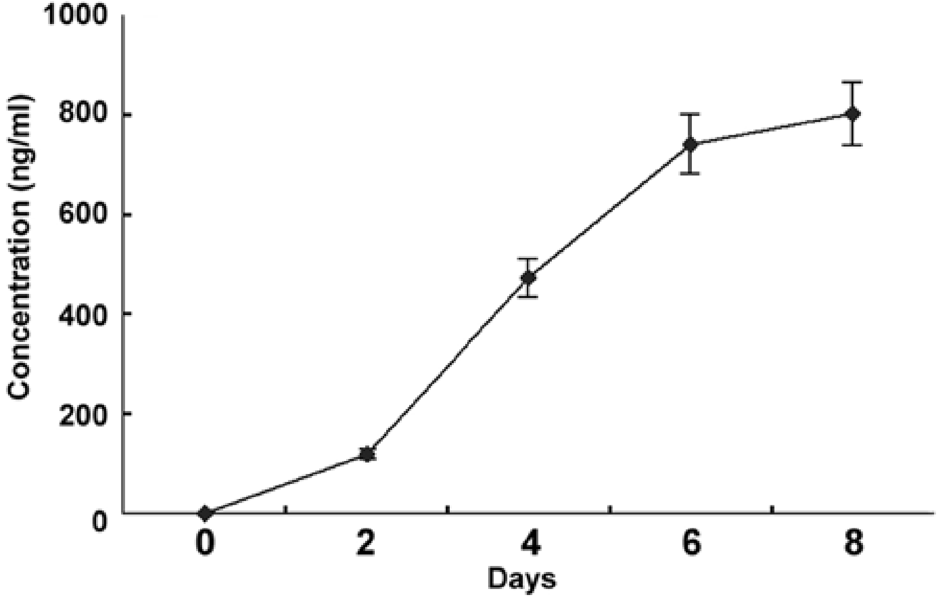




- ZEB2S physically interacts with ZEB2L.
- Complexes of ZEB2L and ZEB2S have lower DNA binding affinity.

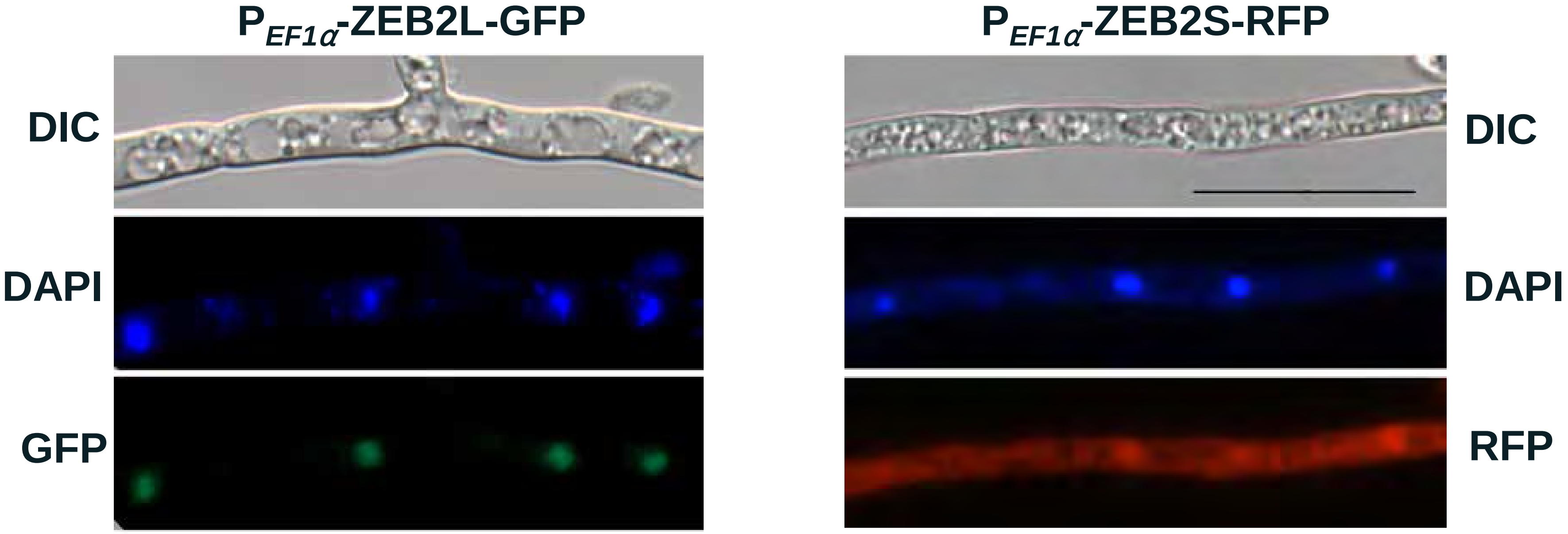
Proposed mechanism of action



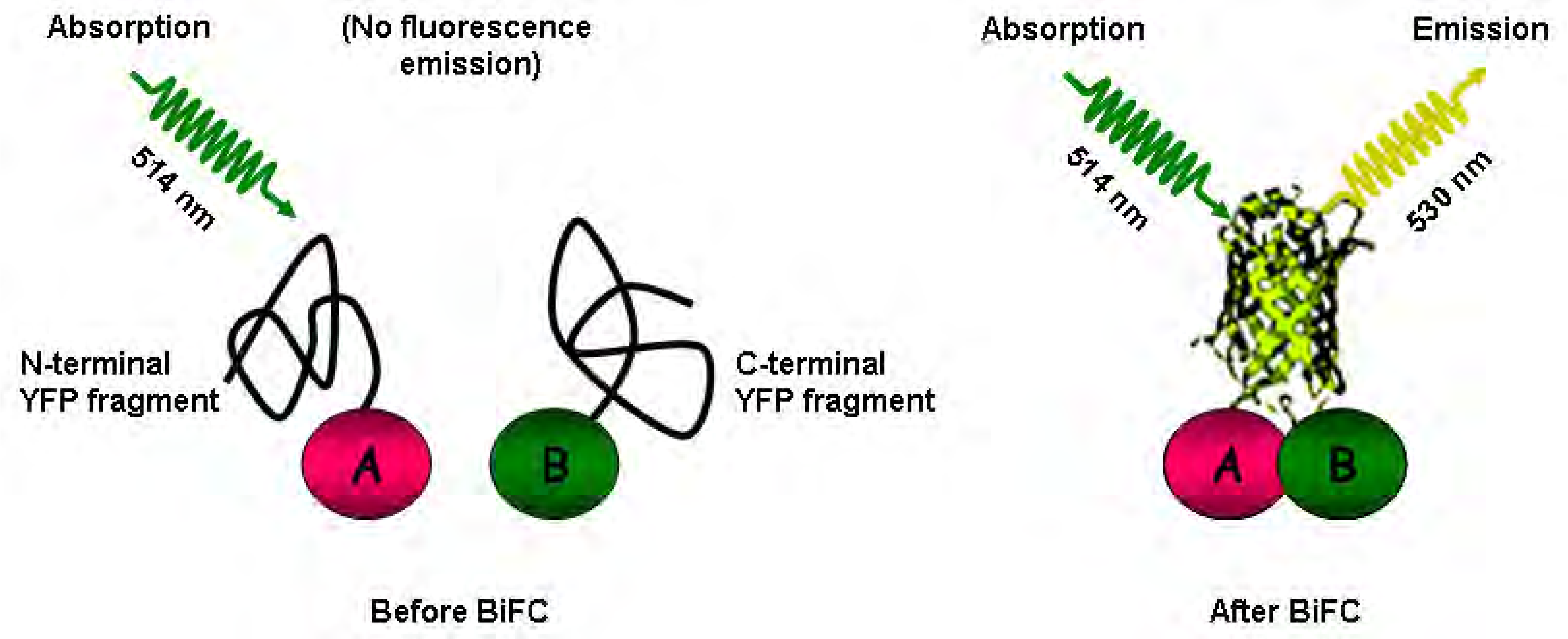


- ZEB2L is a strong activator for ZEA production.
- ZEB2S is an anti-activator of ZEB2L.

Localization two isoforms of ZEB2

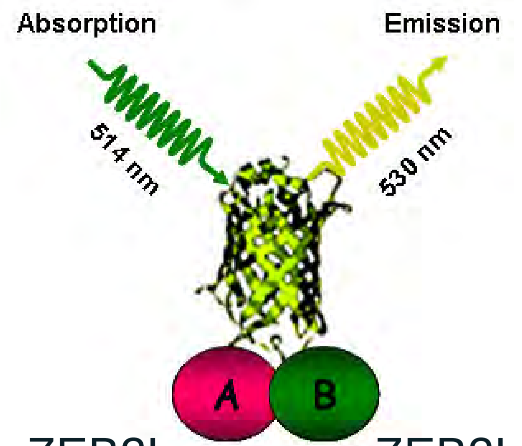


Bimolecular Fluorescence Complementation (BiFC)

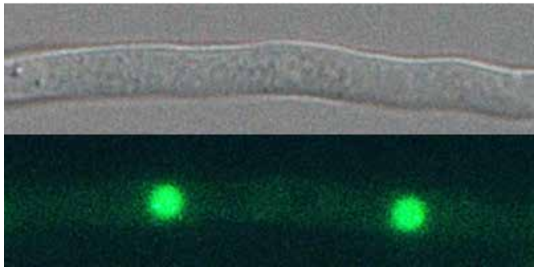


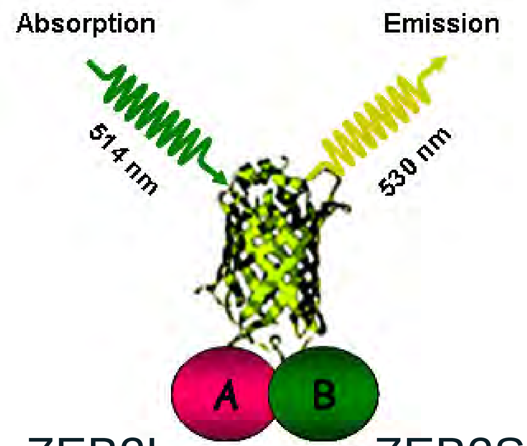
(Bhat et al., 2005, Plant Methods)

Bimolecular Fluorescence Complementation (BiFC)

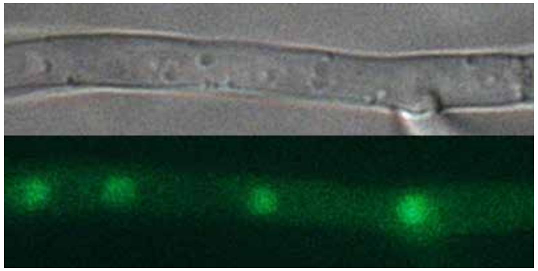


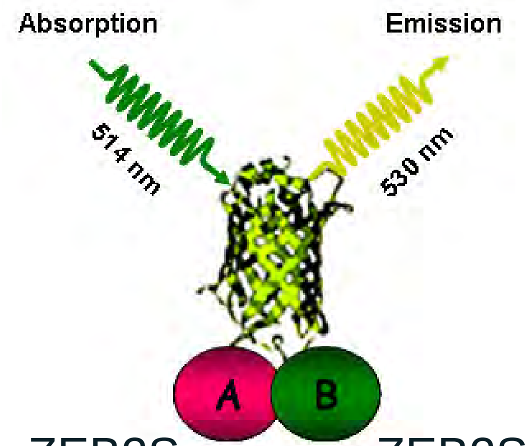
ZEB2L ZEB2L



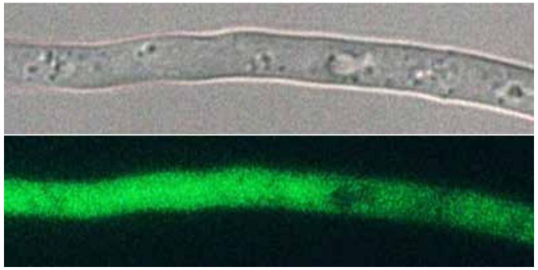


ZEB2L ZEB2S

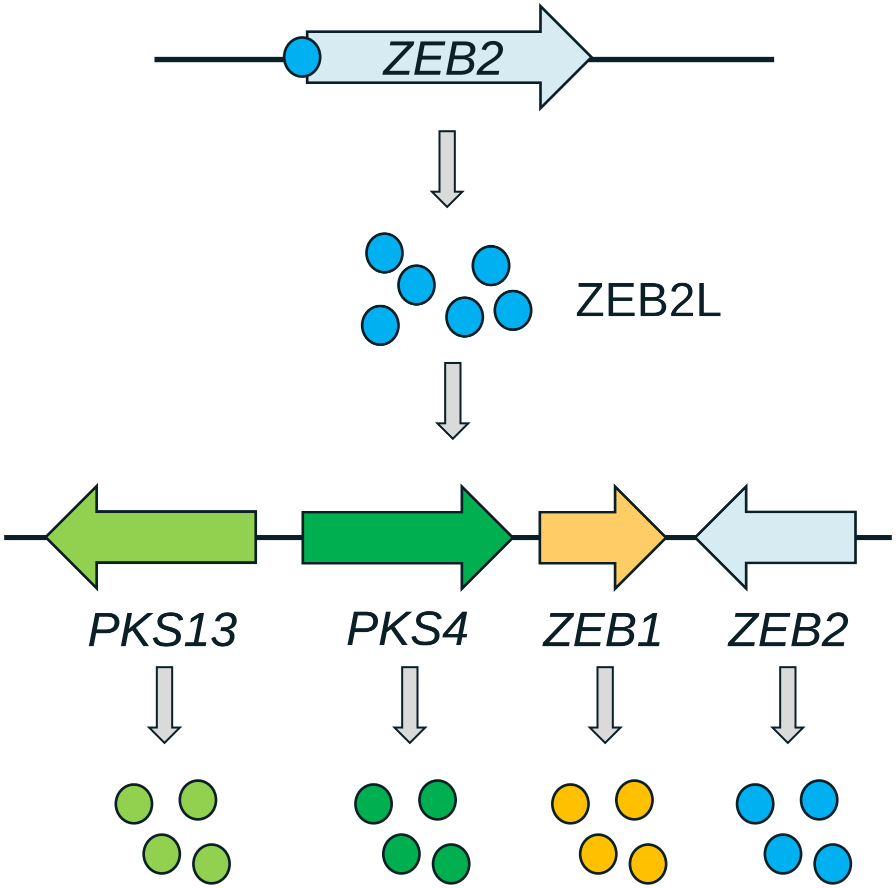




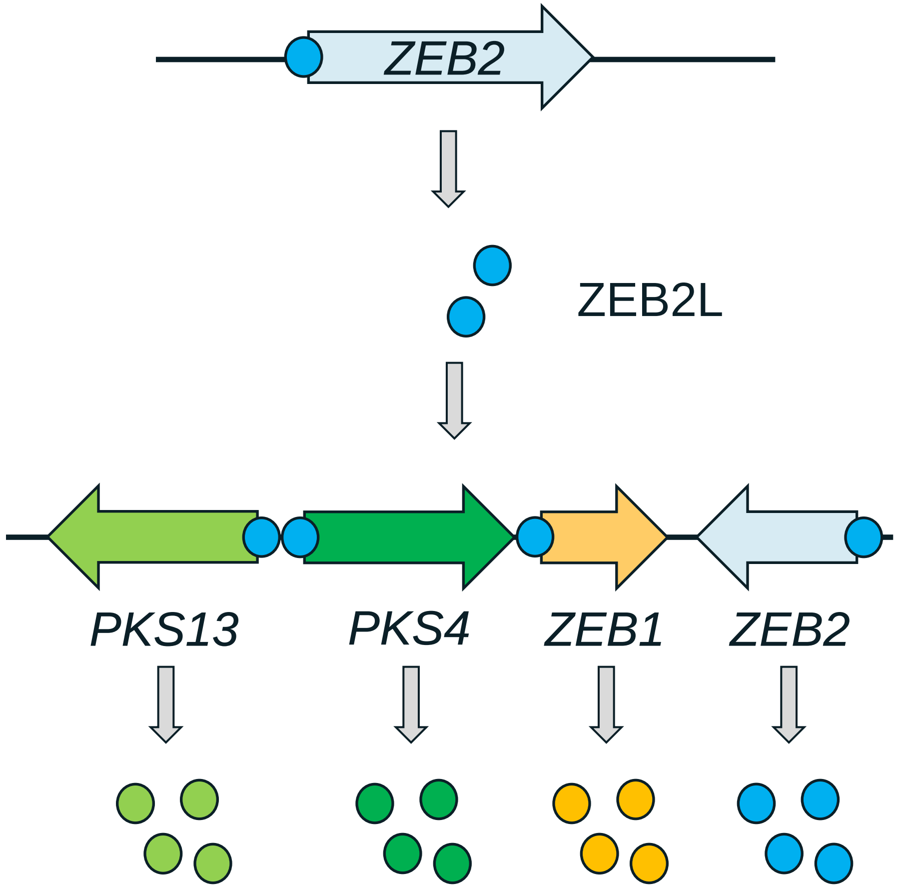
ZEB2S ZEB2S



Proposed mechanism of action

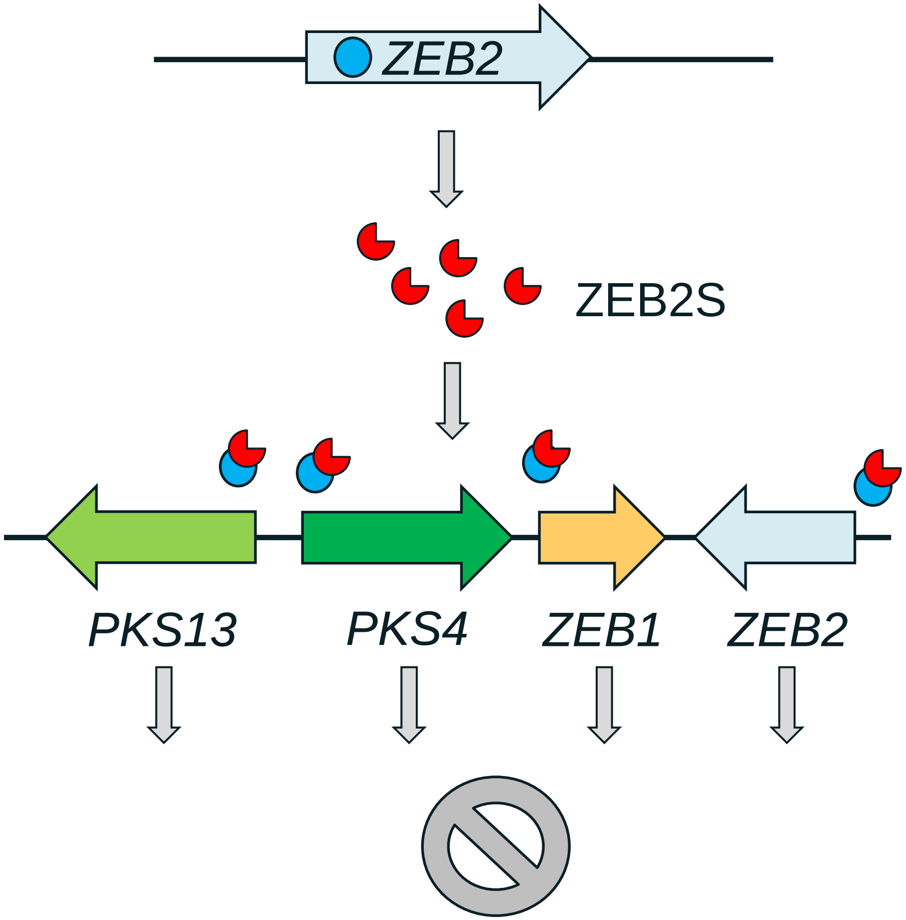


Proposed mechanism of action



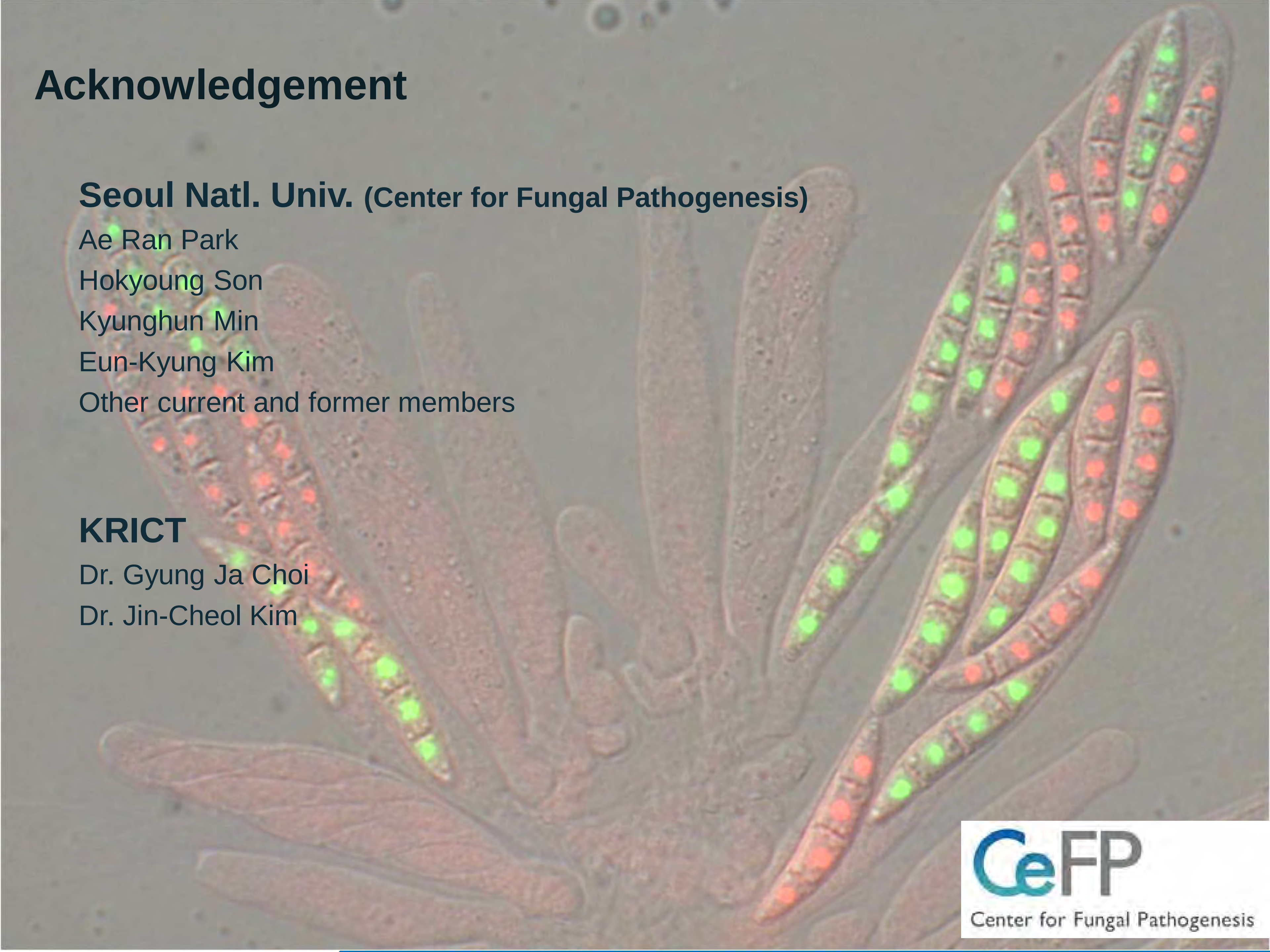
Proposed mechanism of action

High concentration of ZEA or unknown reason



Further works

- Structures of ZEB2 complexes – X-ray crystallography
- Chromatin immunoprecipitation (ChIP) sequencing
- Possible upstream regulator of ZEB2 – Protein kinase A (PKA)
- Biological functions of ZEA?



Acknowledgement

Seoul Natl. Univ. (Center for Fungal Pathogenesis)

Ae Ran Park
Hokyoung Son
Kyunghun Min
Eun-Kyung Kim
Other current and former members

KRICT

Dr. Gyung Ja Choi
Dr. Jin-Cheol Kim