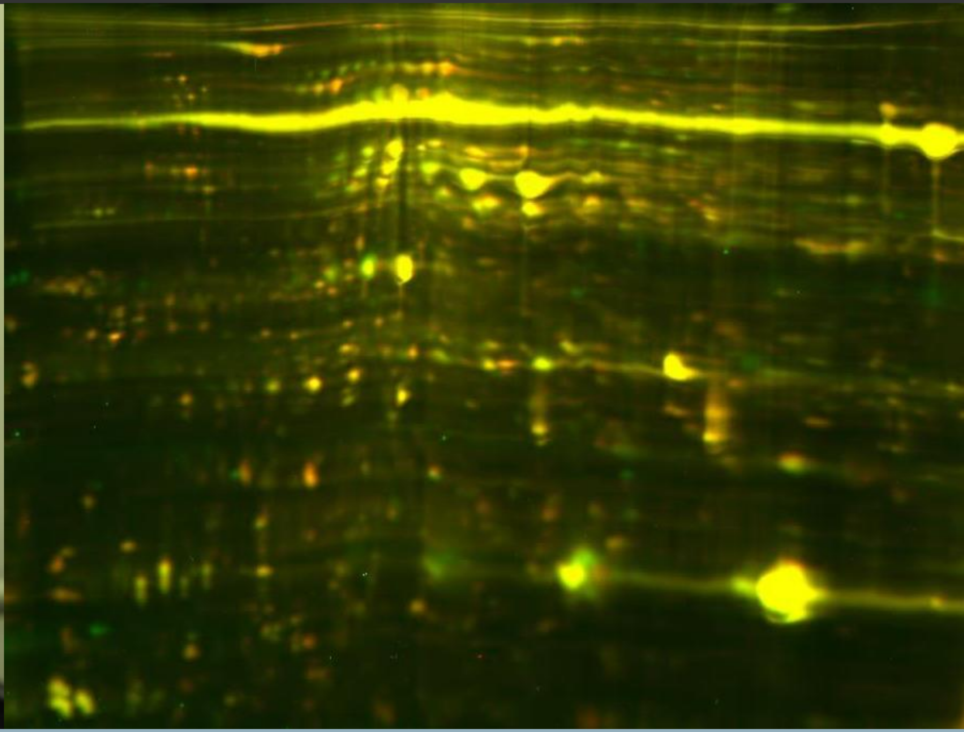


A proteomics approach to dissect *Stagonospora nodorum* effector mode-of-action in wheat

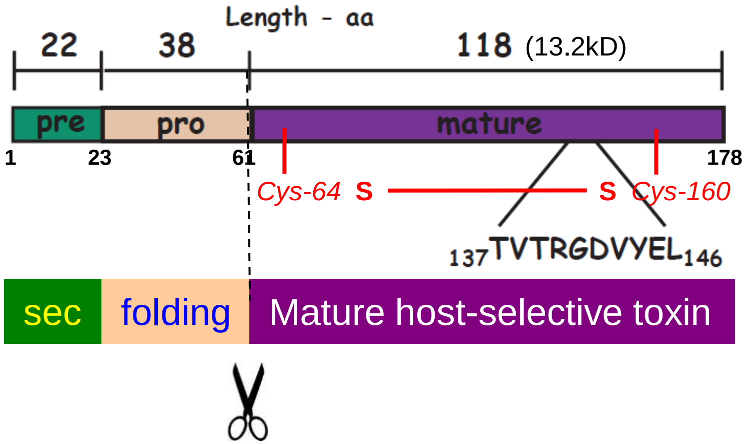
Dr Delphine Vincent

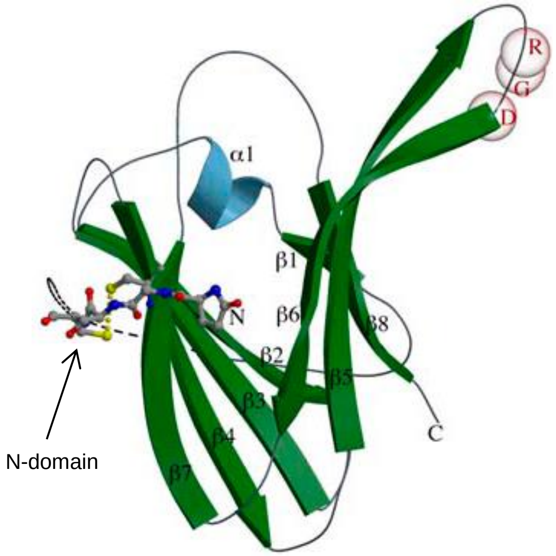
18/03/2011

Introduction

ToxA, the first effector identified in a necroptrophic fungus (*Pyrenophora tritici-repentis*)

1989 1990	ptr necrosis toxin isolated in *P. tritici-repentis* (Ballance et al. 1989; Tomas et al. 1990).
1995	Ptr ToxA = 13.2 kD secreted protein triggering necrosis at 60mM (Tuori et al. 1995)
1997	Ptr ToxA gene cloned (Ciuffetti et al. 1997)
1998	activity requires host metabolism (Kwon et al. 1998).
2000 2002 2004	Cys64-Cys160 (Tuori et al. 2000) RGD (Meinhardt et al. 2002) CK2-like & vitronectin-like (Manning et al. 2004).
2005	crystallography structure (Sarma et al. 2005). internalised by sensitive wheat cells only (Manning et al. 2005).
2007 2008	internalisation through RGD motif (Manning et al. 2008). Targets chloroplast, binds to ToxABP1 (Manning et al. 2007) and PC (Tai et al. 2007).
2009	ROS accumulation and PS alteration (Manning et al. 2009)
2010	ToxA alters ToxABP1 function (Manning et al. 2010).





Ptr ToxA mode-of-action dissected in wheat using transcriptomics

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Tsn1-mediated host responses to ToxA from *Pyrenophora tritici-repentis*.

Adhikari TB, Bai J, Meinhardt SW, Gurung S, Myrfield M, Patel J, Ali S, Gudmestad NC, Rasmussen JB

MPMI 2009, *22 (9): 1056-68*

Time course: 0, 0.5, 4, 12, 24, 48 hpi

Affymetrix GeneChip Wheat Genome Array

Induction of signalling events and lignification, and production of reactive oxygen species

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Analysis of transcriptome changes induced by Ptr ToxA in wheat provides insights into the mechanisms of plant susceptibility.

Pandelova I, Betts MF, Manning VA, Wilhelm LJ, Mockler TC, Ciuffetti LM

Molecular Plant 2009, *2 (5): 1067-83*

Time course: 0, 3, 9, 14 hpi

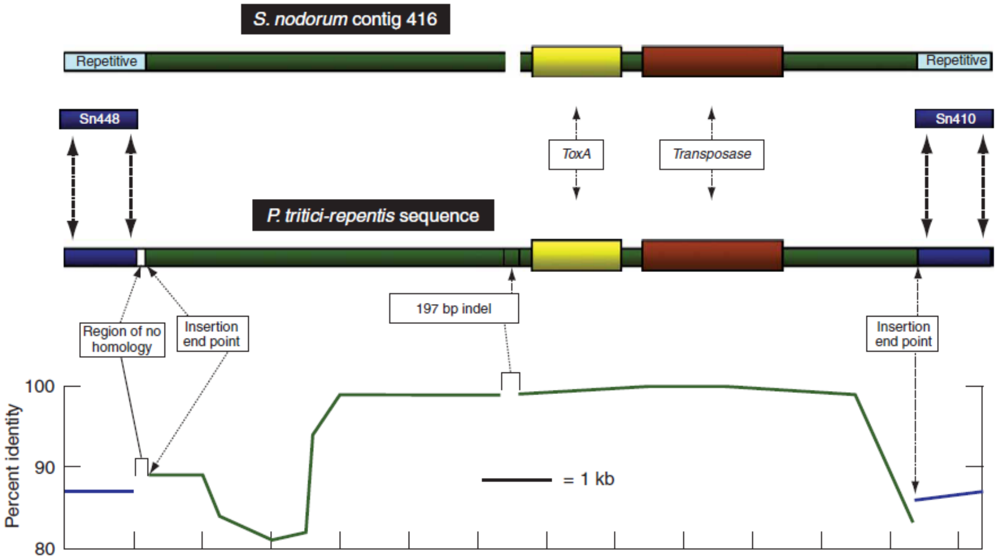
Affymetrix GeneChip Wheat Genome Array

Impairment of the photosynthetic machinery and accumulation of reactive oxygen species

SnToxA/Tsn1: the wheat susceptibility gene

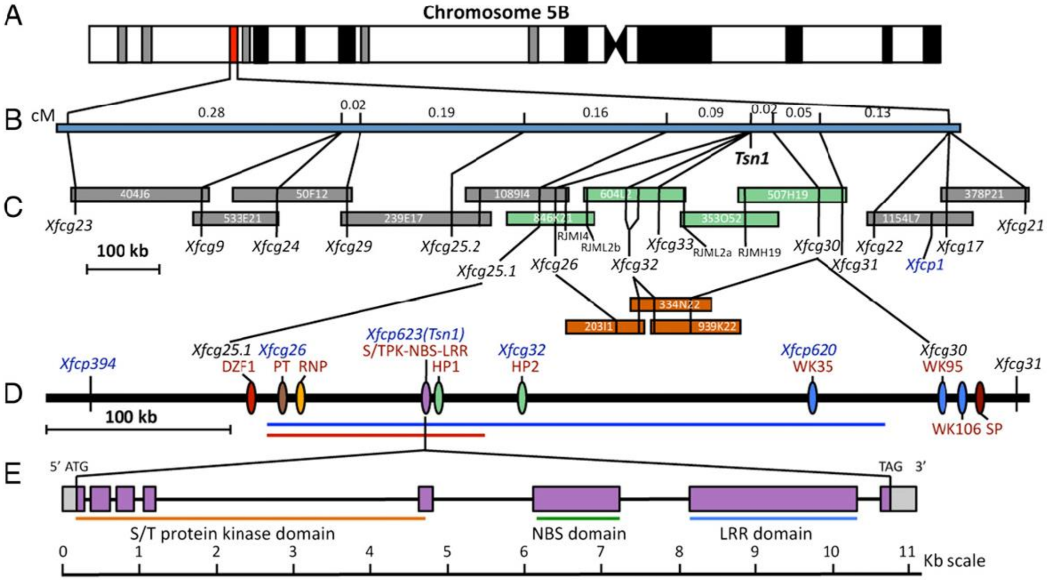
2006

Stagonospora nodorum closely related to *P. tritic-repentis*.
ToxA exists in *S. nodorum* (SnToxA) and is 99.7% similar to Ptr ToxA.
Ptr ToxA was acquired from SnToxA through HGT (Friesen et al. 2006).

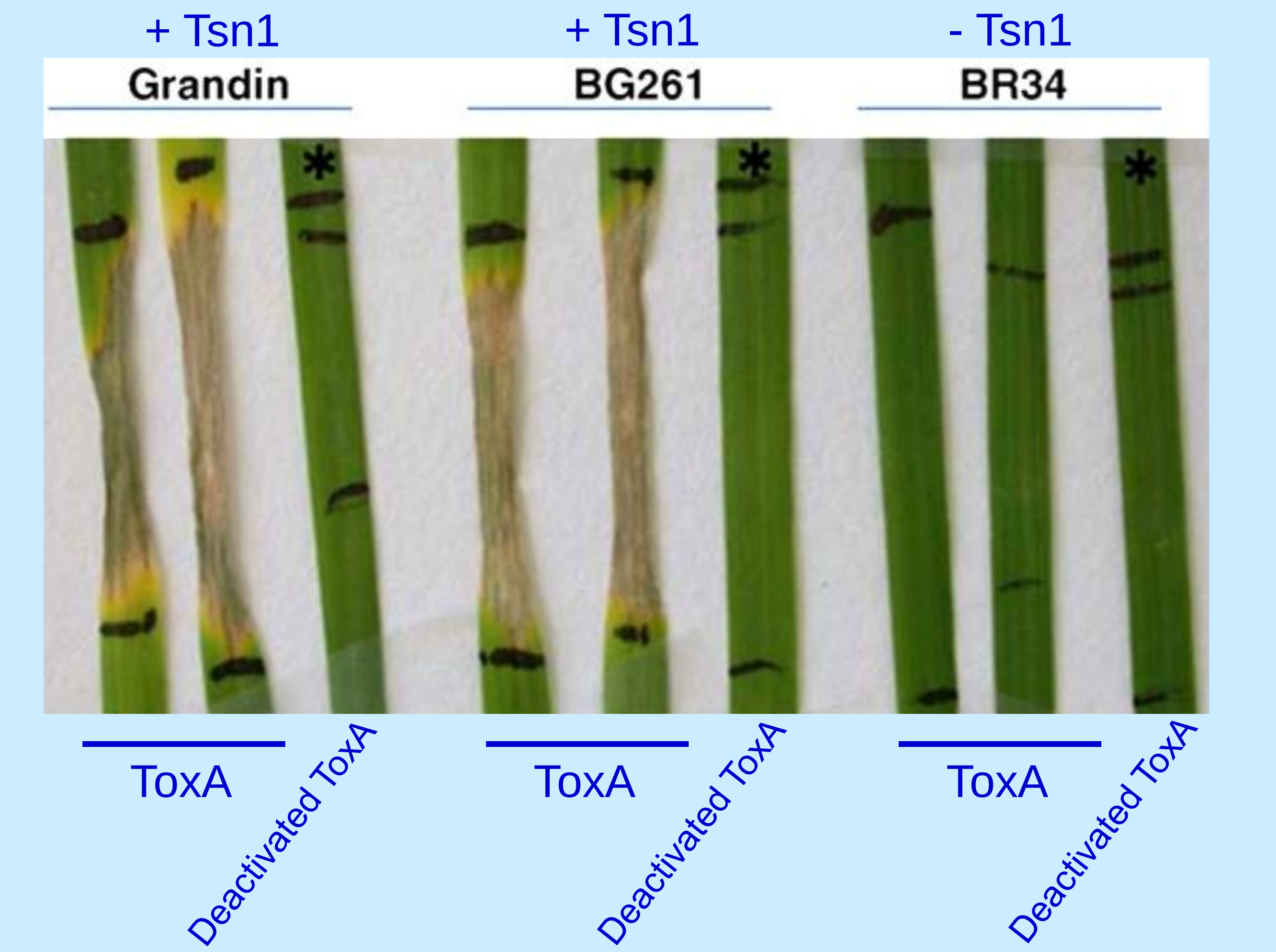


2010

Tsn1 gene cloned (Faris et al. 2010).
- -located on chromosome 5BL
- -features of a R gene
- -not a receptor of ToxA

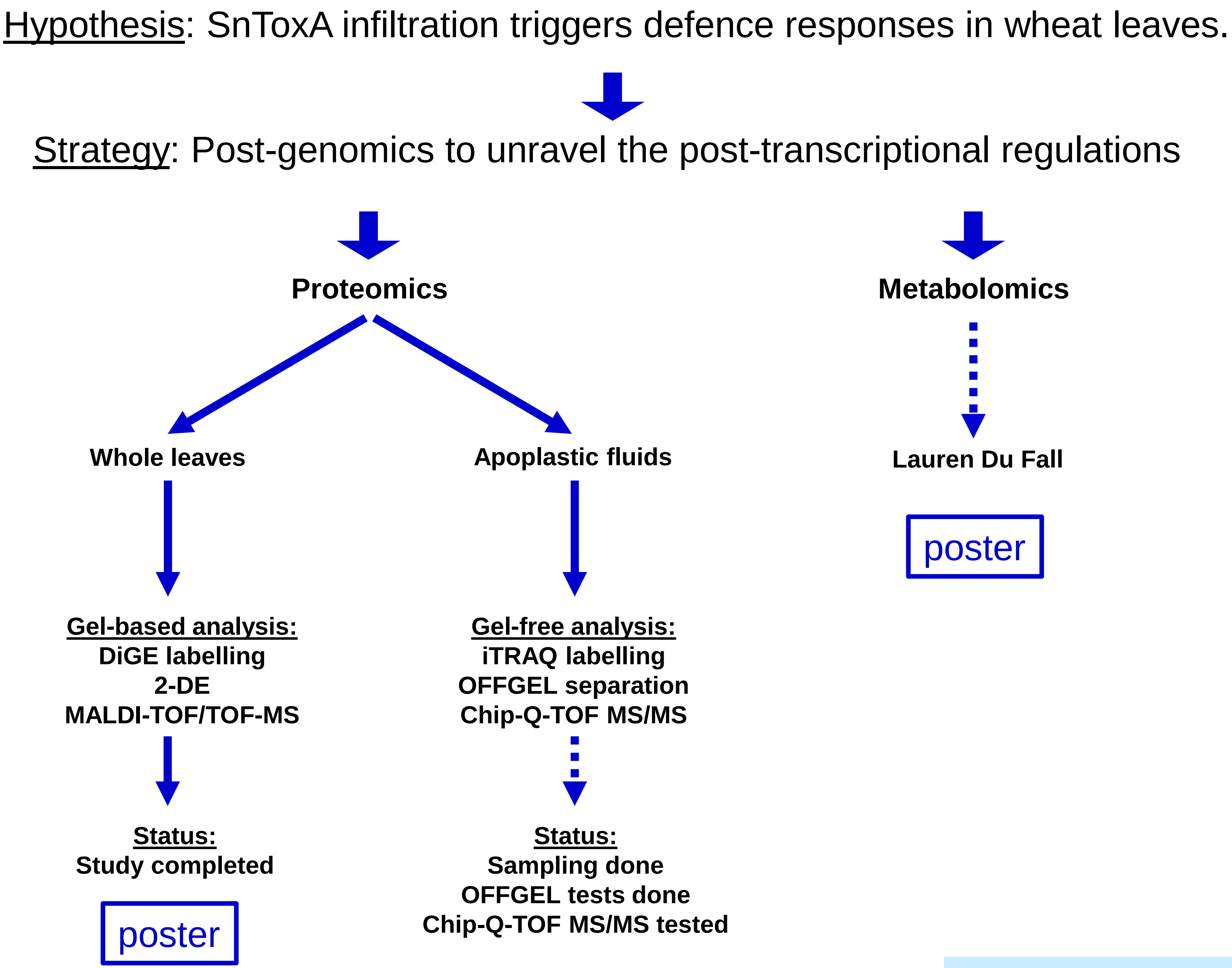


ToxA/Tsn1: inverse gene-for-gene system, *i.e.* Effector Triggered Susceptibility (ETS)



(Oliver & Solomon, 2010)

Strategy



Hypothesis: SnToxA infiltration triggers defence responses in wheat leaves.

Strategy: Post-genomics to unravel the post-transcriptional regulations

Proteomics

Whole leaves

Gel-based analysis:
DiGE labelling
2-DE
MALDI-TOF/TOF-MS

Status:
Study completed

poster

Apoplastic fluids

Gel-free analysis:
iTRAQ labelling
OFFGEL separation
Chip-Q-TOF MS/MS

Status:
Sampling done
OFFGEL tests done
Chip-Q-TOF MS/MS tested

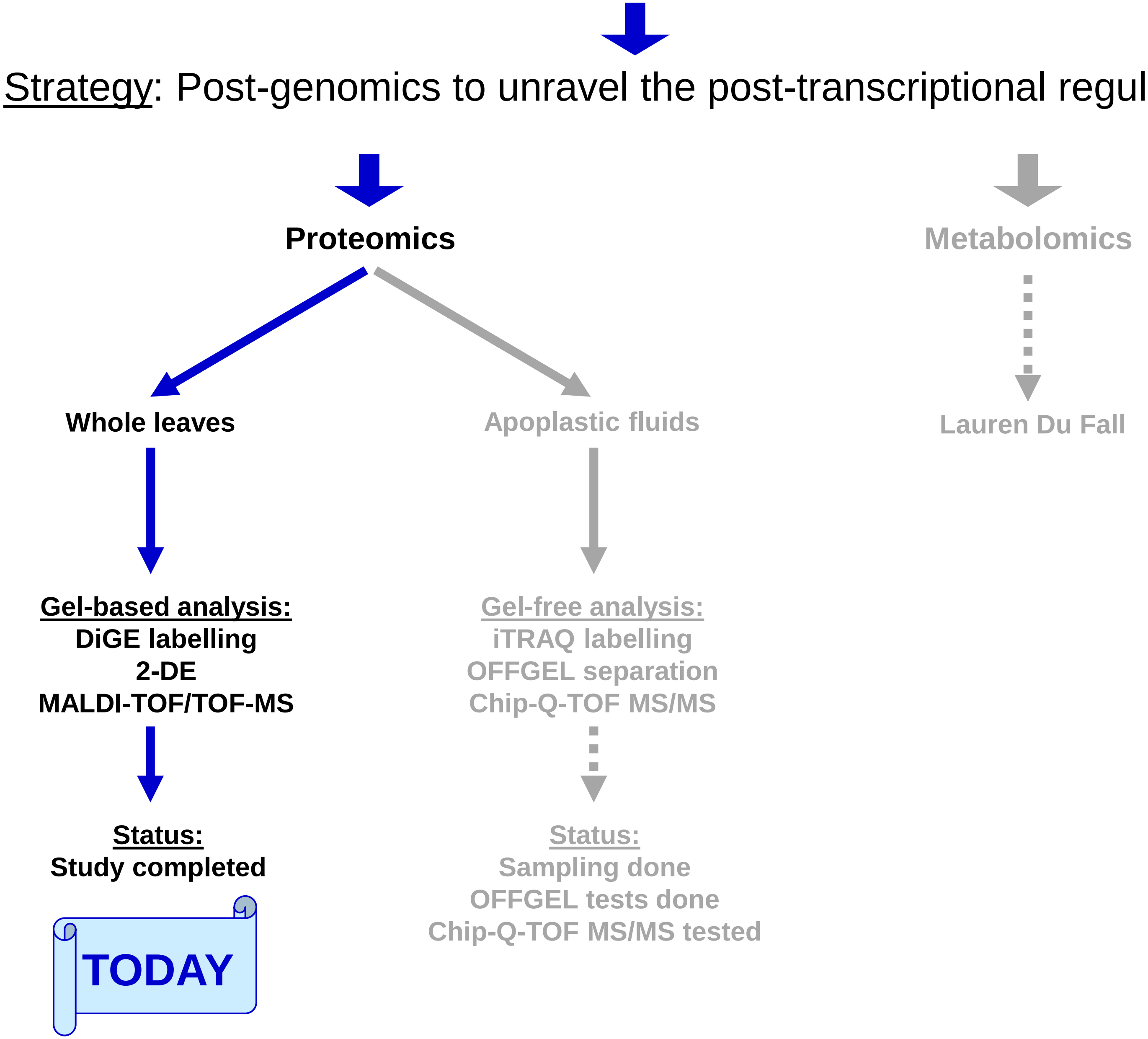
Metabolomics

Lauren Du Fall

poster

Proteome=set of all proteins
Metabolome=set of all metabolites

Hypothesis: SnToxA infiltration triggers defence responses in wheat leaves.



Strategy: Post-genomics to unravel the post-transcriptional regulations

Proteomics

Metabolomics

Whole leaves

Apoplastic fluids

Lauren Du Fall

Gel-based analysis:
DiGE labelling
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MALDI-TOF/TOF-MS

Gel-free analysis:
iTRAQ labelling
OFFGEL separation
Chip-Q-TOF MS/MS

Status:
Study completed

TODAY

Status:
Sampling done
OFFGEL tests done
Chip-Q-TOF MS/MS tested

Materials

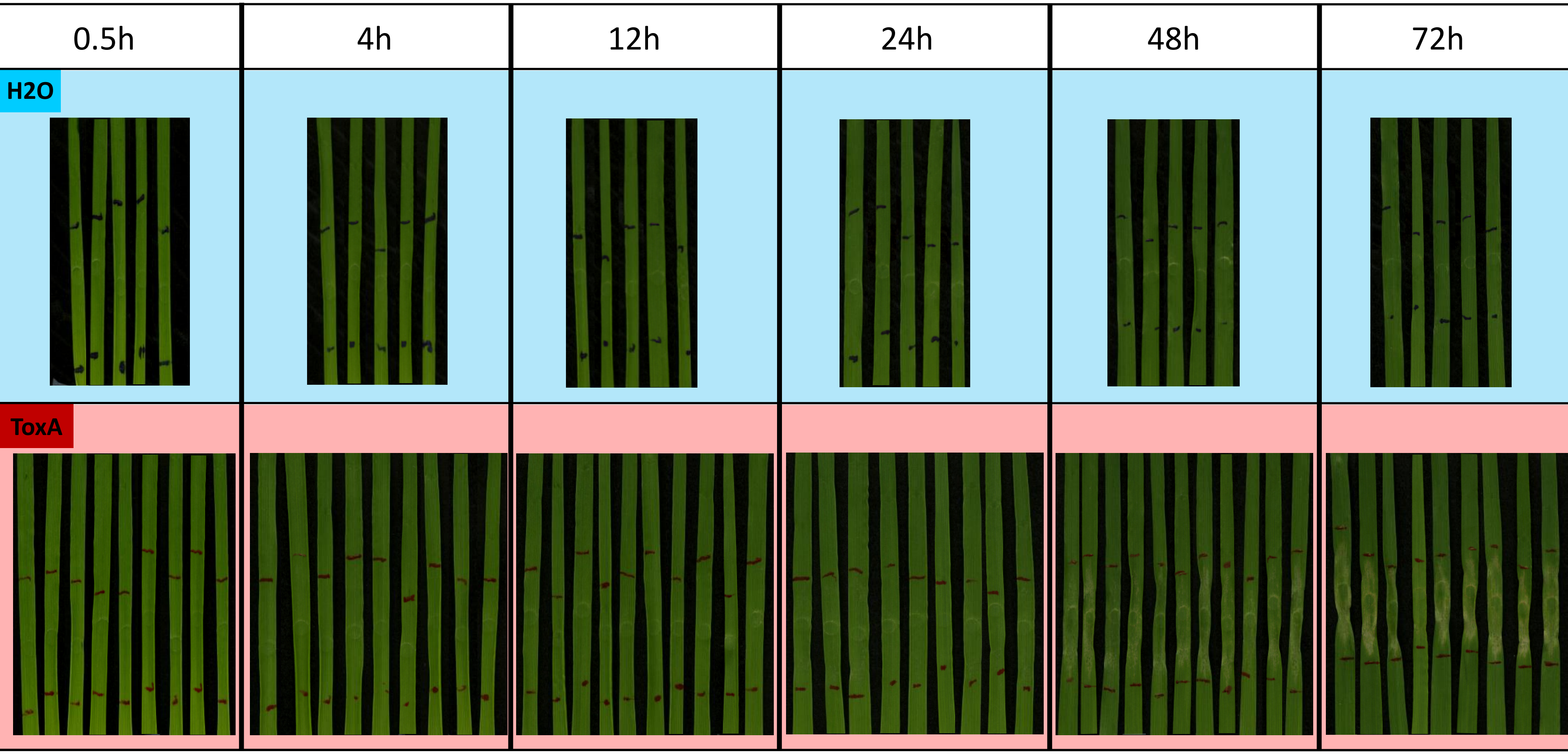
Flowchart of the experimental design for gel-based proteomics

Plant culture (*T. aestivum* cv. BG261) & leaf infiltration in growth room

Post-infiltration leaf sampling over time course:

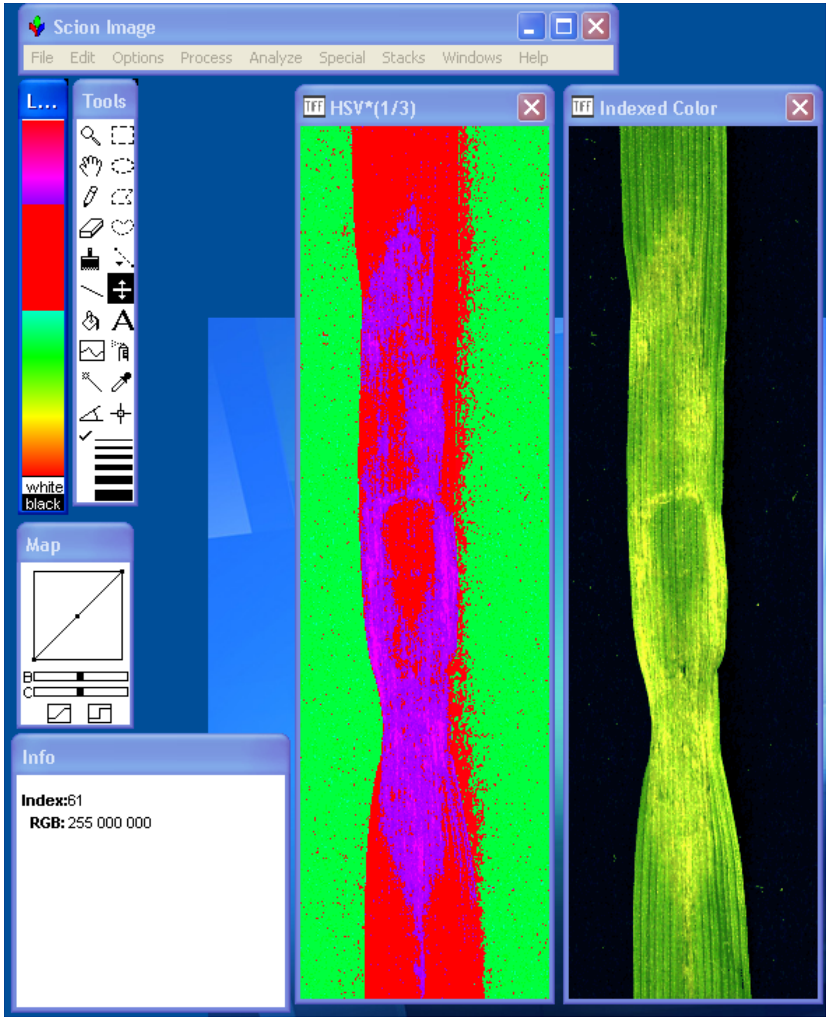
2 treatments (H_2O, ToxA)
6 time points (0, ½, 4, 12, 24, and 48h) (Adhikari et al. 2009)
3 biological replicates (1BL=pool of 10 leaves)
→ 36 samples

Quantitation of ToxA symptoms



Gradual appearance of the symptoms.
Obvious yet subtle chlorosis/necrosis after 48h.
Tissues not too damaged for protein extraction.

Quantitation of ToxA symptoms



Index:61
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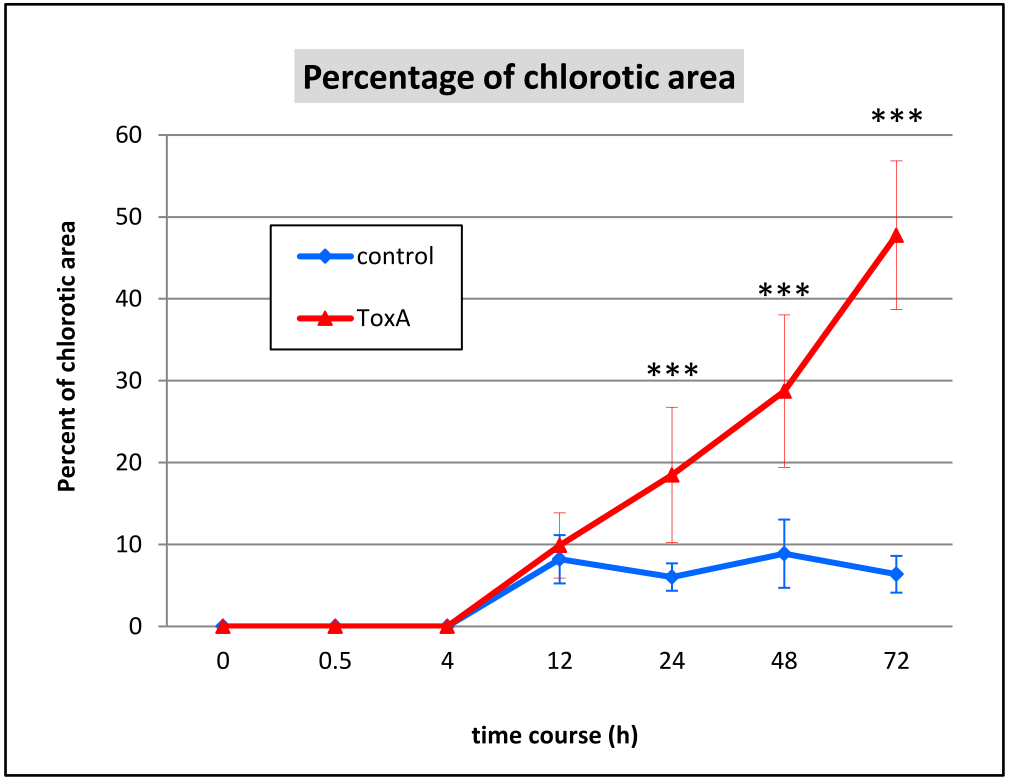
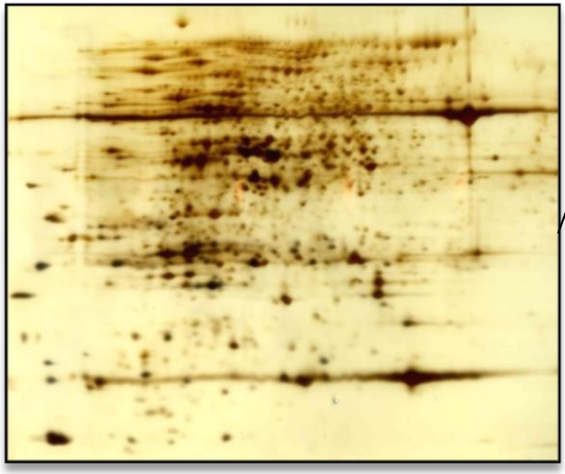
Quantifying fungal infection of plant leaves by digital image analysis using Scion image software.
Wijekoon CP, Goodwin PH, Hsiang T.
J Microbiol Meth 2008, 74:94-101



Accurate quantitation of symptoms.
Infiltration damage starts to be visible after 12h and is stable over time (blue curve).
ToxA symptoms become noticeable after 24h and steadily worsen (red curve).

Methods

Flowchart of the experimental design for gel-based proteomics



Plant culture (*T. aestivum* cv. BG261) & leaf infiltration in growth room

↓

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3 biological replicates (1BL=pool of 10 leaves)
→ 36 samples

↓

Extraction of soluble proteins

↓

Protein abundances determined using 2-DE

↙ ↘

	Acidic proteins	Basic proteins
Samples	36	12 (24/48h)
Protein load	0.1 mg	0.5 mg
pH range	4-7	7-10
Labelling	DiGE minimal kit	none

↓

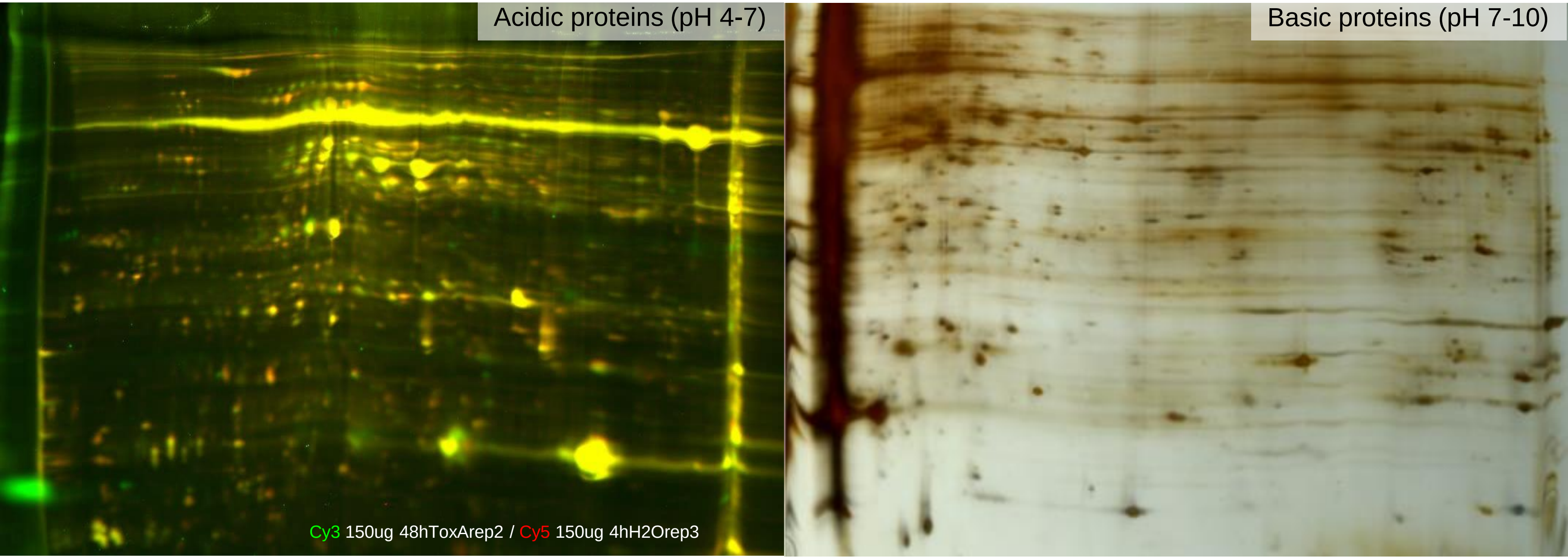
Differentially-regulated proteins analysed using statistical analyses

↓

Identification of significant spots by mass spectrometry (MS)

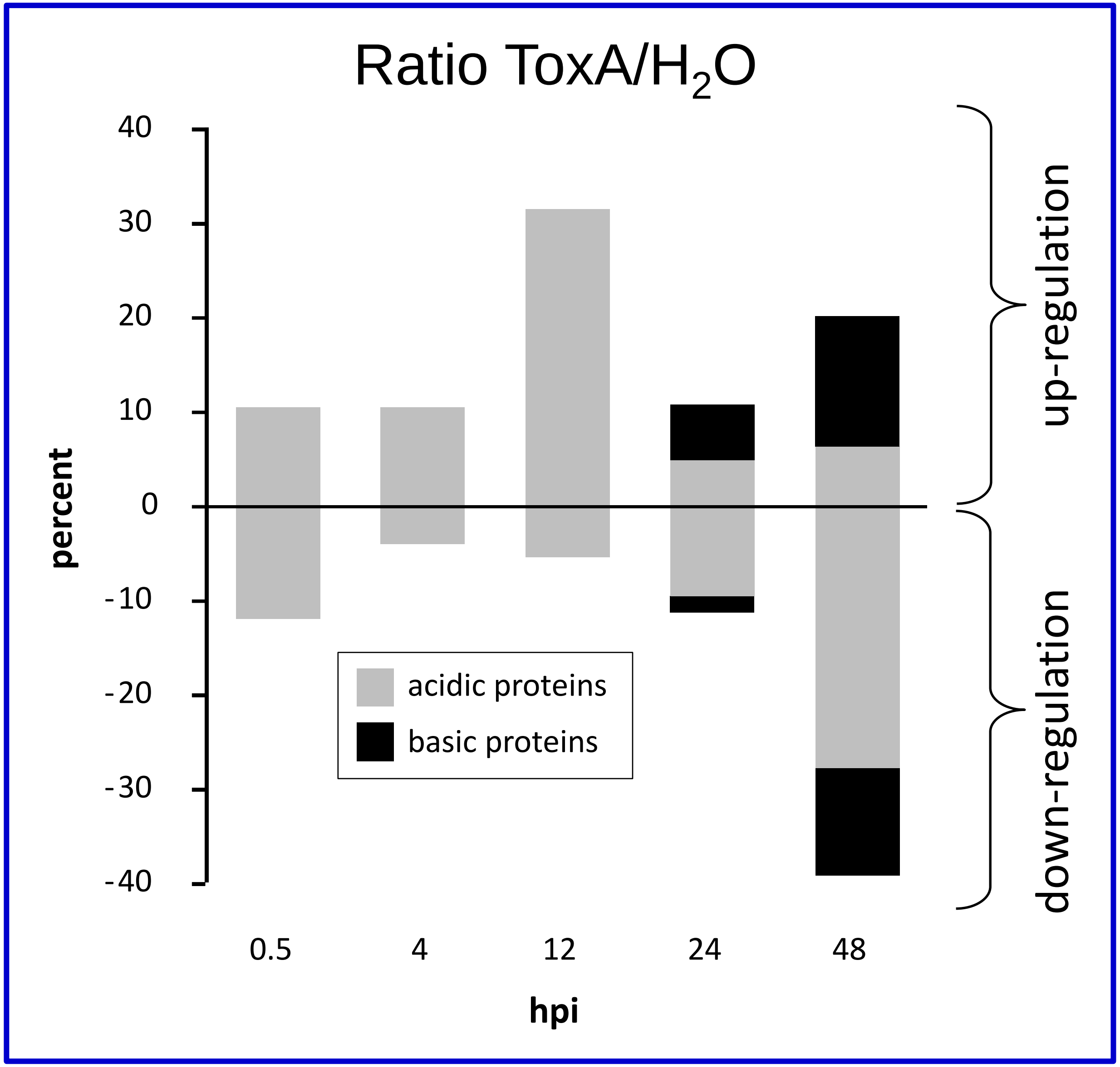
Results

Proteomic analysis using two-dimensional electrophoresis



	Acidic range	Basic range	TOTAL
Detected spots	1070	530	1600
Significant spots (2-way ANOVA)	294	72	366
Identified spots (MS/MS)	70/76	40/54	110/130
Unique description	46	38	81 (3 shared)
Unknown	10	6	16
Chloroplastic (ChloroP, literature)	57	18	75

Protein expression profiling



Most of the differences occur at 48 hpi.

Main trend:
- induction at 12 hpi
- repression at 48 hpi

Comparison with other pathosystems

► wheat leaves infected with *Puccinia triticina* (Rampitsch et al., 2006):

14-3-3 proteins,
peptidyl-prolyl cis-trans isomerases,
elongation factors 1b,
70 kD heat shock proteins,
Cp31BHv nucleic acid-binding proteins,
cytosolic triosephosphate isomerase.

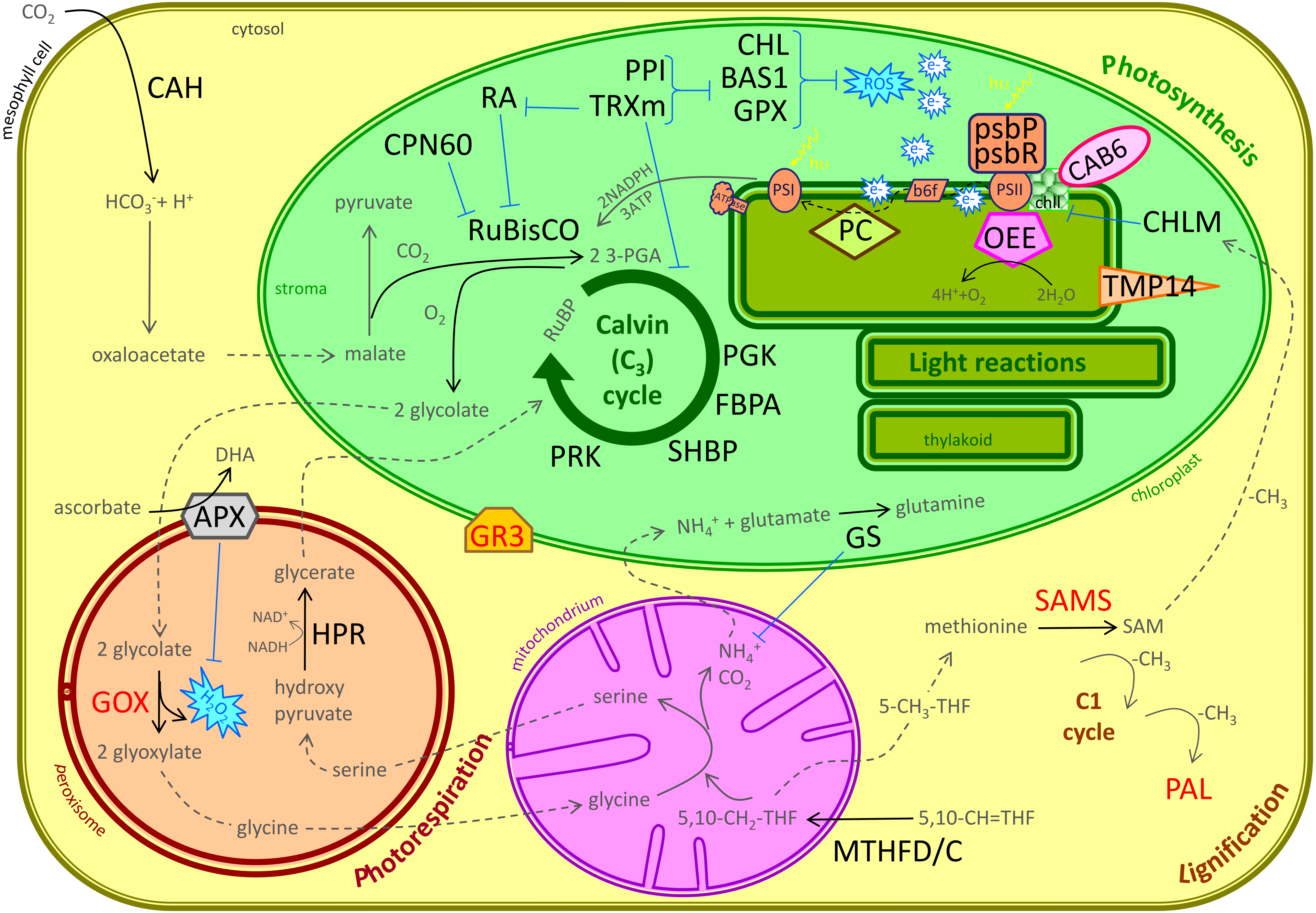
► wheat spikes infected with *Fusarium graminearum* (Wang et al., 2005; Zhou et al., 2006; Dornez et al., 2010):

90 kD heat shock proteins,
thioredoxin m,
ascorbate peroxidase,
RuBisCO,
RuBisCO activase,
fructose-bisphosphate aldolase

The induction of host proteins interacting with fungal components in our system which lacks the pathogen highlights the ability of SnToxA to tricking the host in believing in a pathogen attack and initiating a hypersensitive response (HR) leading to cell death.

HR would be mediated by an early antioxidant mechanism (within 24h) and a late PR-protein induction as well as photosynthesis and photorespiration collapse (at 48 hpi).

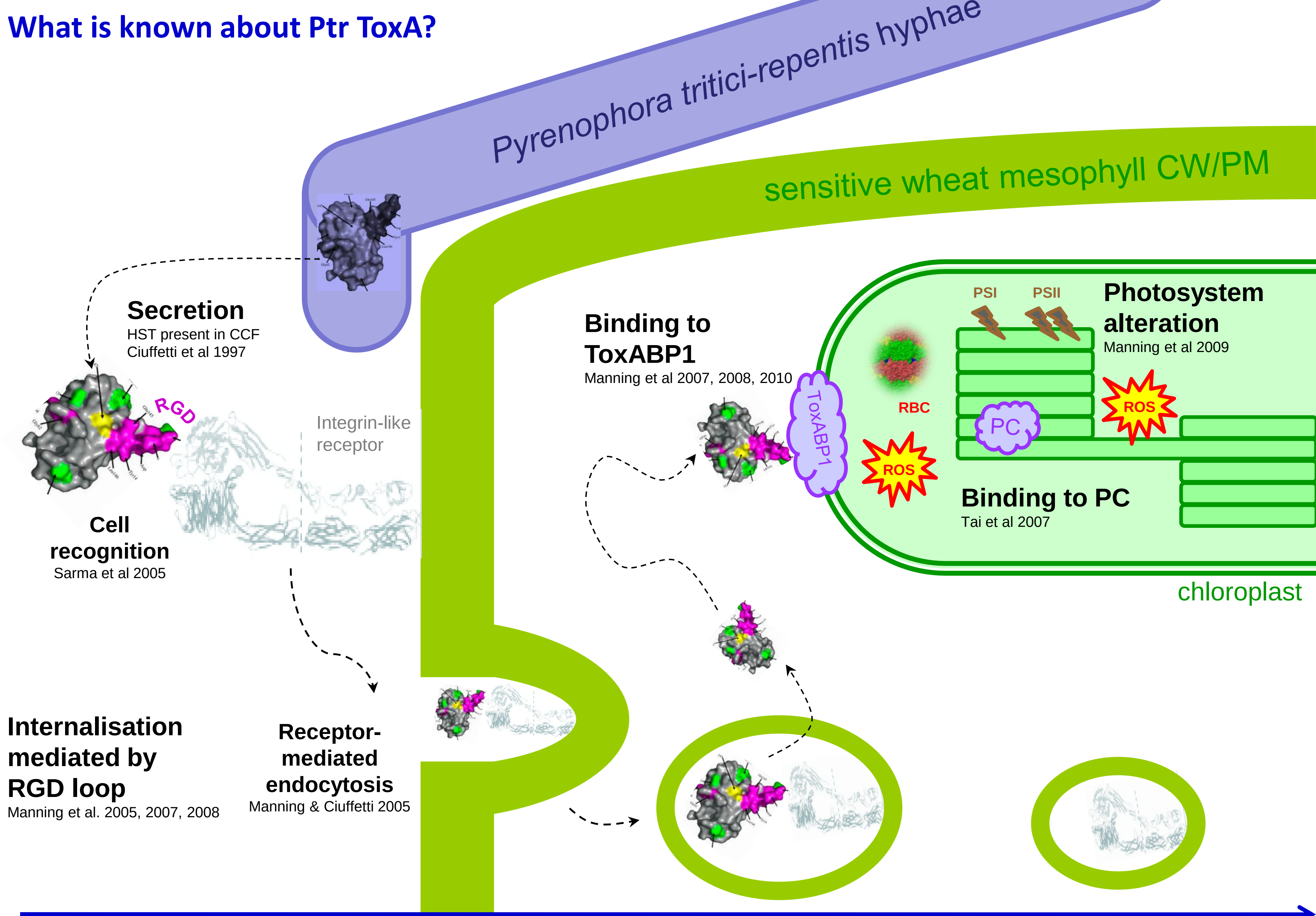
Putative roles of ToxA-responsive proteins in wheat mesophyll cells



CO_2
mesophyll cell
cytosol
CAH
HCO_3^-+ H^+
oxaloacetate
malate
pyruvate
CO_2
O_2
stroma
CPN60
RA
PPI
TRXm
CHL
BAS1
GPX
ROS
e-
RuBisCO
2NADPH
3ATP
2 3-PGA
RuBP
Calvin (C_3) cycle
PGK
FBPA
SHBP
PRK
2 glycolate
Photosynthesis
hυ
psbP
psbR
CAB6
PSI
ATPase
b6f
PSII
chll
PC
OEE
CHLM
$4H^+$+O_2
$2H_2O$
TMP14
Light reactions
thylakoid
chloroplast
GR3
NH_4^+ + glutamate
glutamine
GS
DHA
ascorbate
APX
glycerate
NAD^+
NADH
HPR
2 glycolate
GOX
H_2O_2
2 glyoxylate
hydroxy pyruvate
serine
glycine
peroxisome
Photorespiration
mitochondrium
serine
glycine
NH_4^+
CO_2
5,10-CH_2-THF
5,10-CH=THF
MTHFD/C
5-CH_3-THF
methionine
SAMS
SAM
-CH_3
-CH_3
-CH_3
C1 cycle
PAL
Lignification

Working model

What is known about Ptr ToxA?



Secretion
HST present in CCF
Ciuffetti et al 1997

Cell recognition
Sarma et al 2005

Internalisation mediated by RGD loop
Manning et al. 2005, 2007, 2008

Receptor-mediated endocytosis
Manning & Ciuffetti 2005

Binding to ToxABP1
Manning et al 2007, 2008, 2010

Photosystem alteration
Manning et al 2009

Binding to PC
Tai et al 2007

Immediate interaction with high affinity receptor

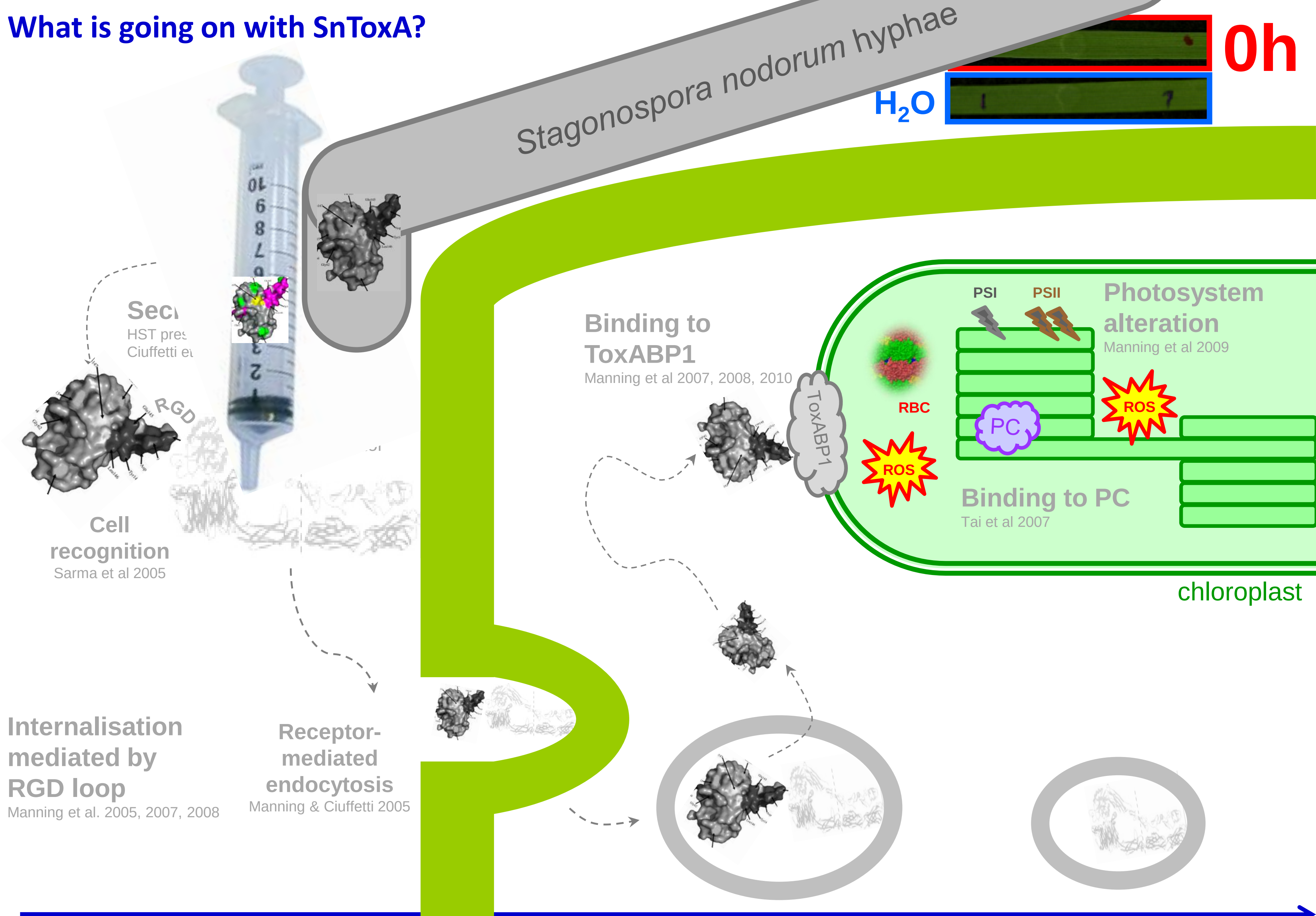
Internalisation 2h

Chloroplast localisation 14h

ROS release 18h

Necrosis 24h

What is going on with SnToxA?



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HST pres
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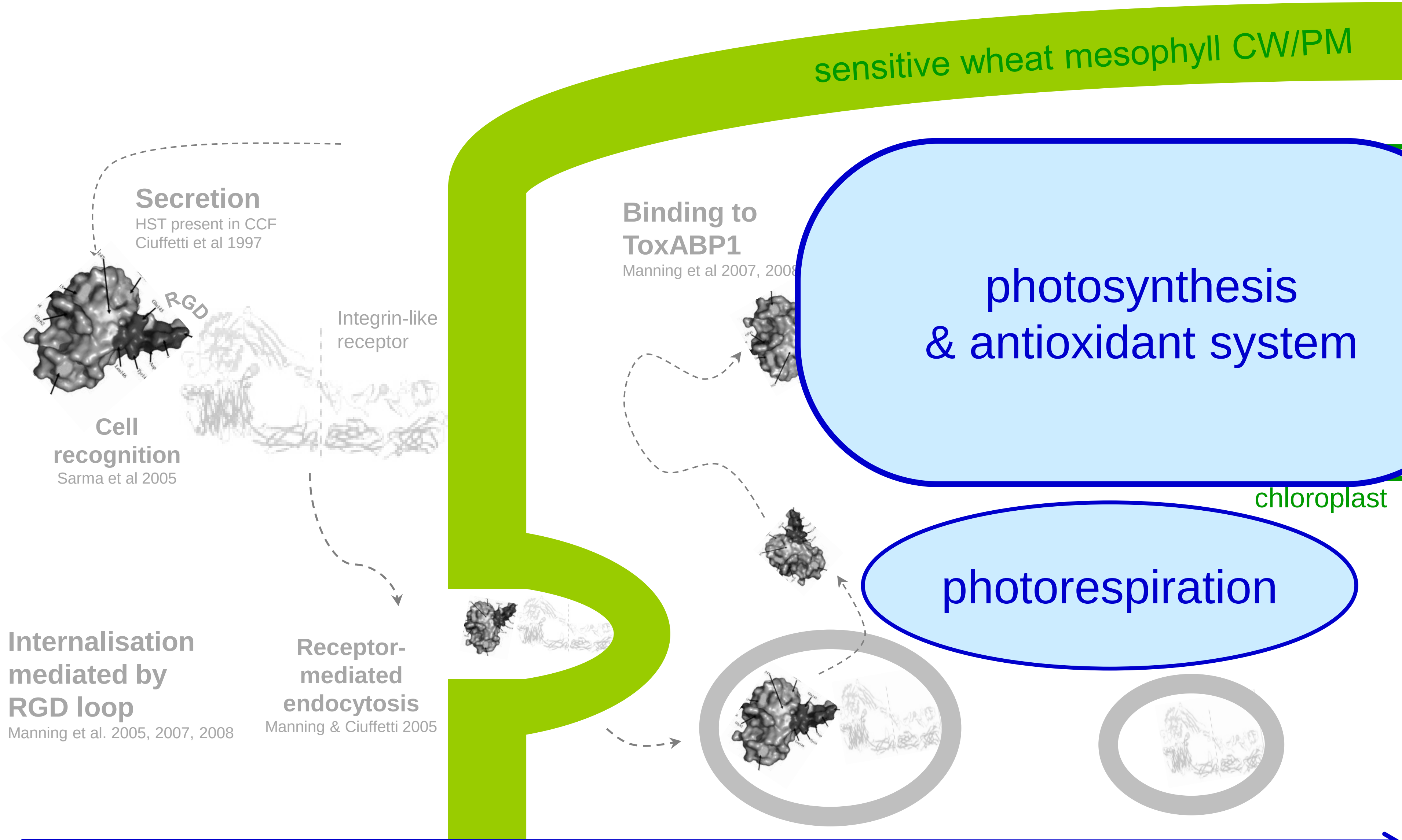

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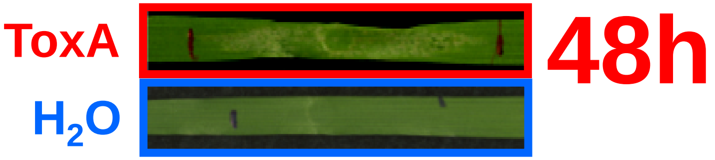

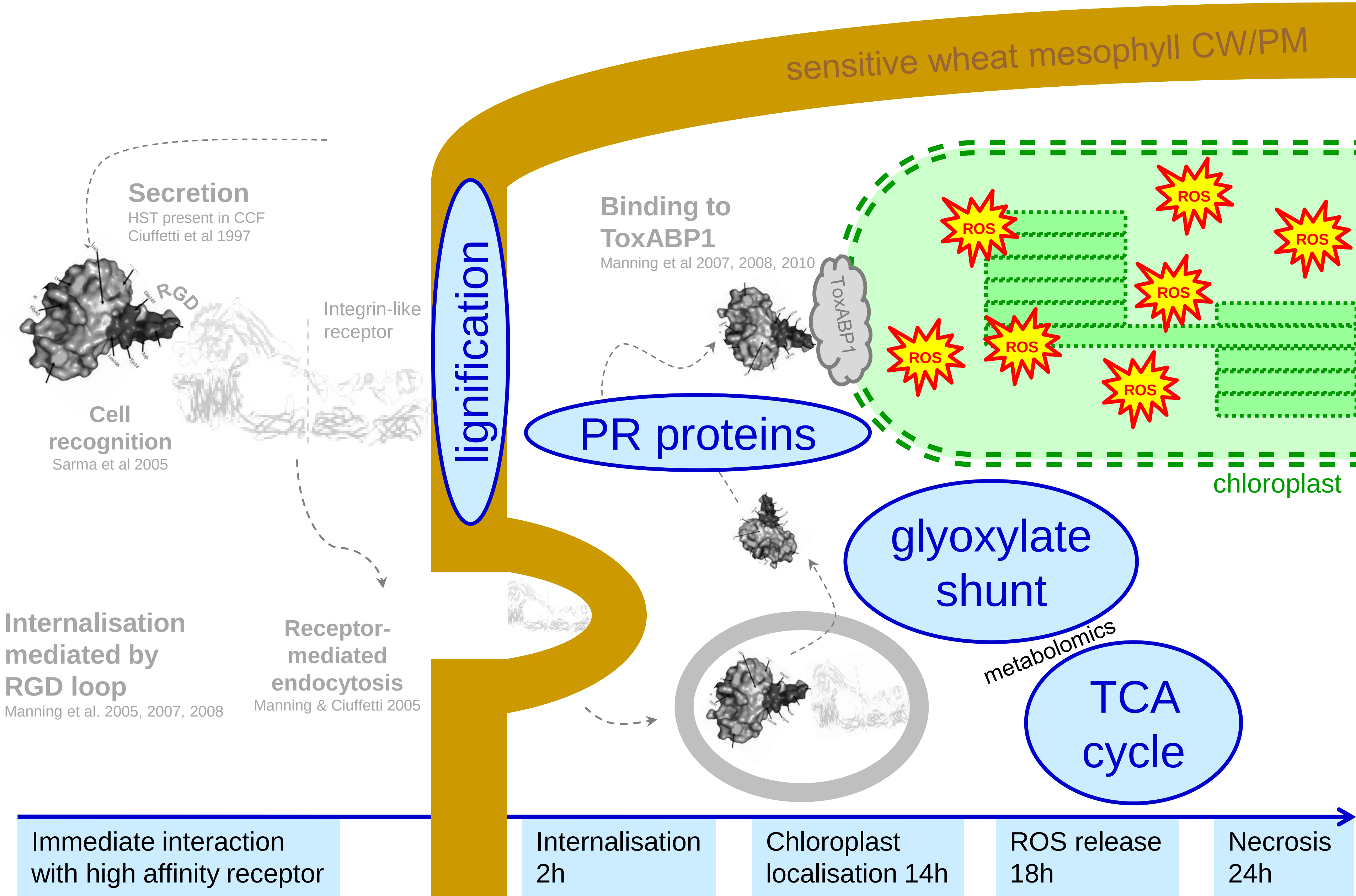

Secretion
HST present in CCF
Ciuffetti et al 1997

Cell recognition
Sarma et al 2005

Integrin-like receptor

Binding to ToxABP1
Manning et al 2007, 2008, 2010

PR proteins

glyoxylate shunt

TCA cycle

Internalisation mediated by RGD loop
Manning et al. 2005, 2007, 2008

Receptor-mediated endocytosis
Manning & Ciuffetti 2005

Immediate interaction with high affinity receptor

Internalisation 2h

Chloroplast localisation 14h

ROS release 18h

Necrosis 24h

Conclusions

Future directions

Proteomics + metabolomics (complementary techniques) complete the mode-of-action picture drawn from transcriptomics.

Another finding is that the drop in carbohydrate and ATP supplies due to photosynthesis collapse (proteomics) seems to be salvaged, to a limited extent, by enhanced TCA cyle and glyoxylate shunt (metabolomics).

This proof-of-concept study helped us establishing post-genomics techniques never used before in this system.

The same strategy will be applied to study the mode-of-actions of Tox3 and Tox1, other effectors of *S. nodorum*.

Thank you !