THE SAINSBURY LABORATORY

Lessons in effector and NLR biology of plant-microbe systems

Sophien Kamoun

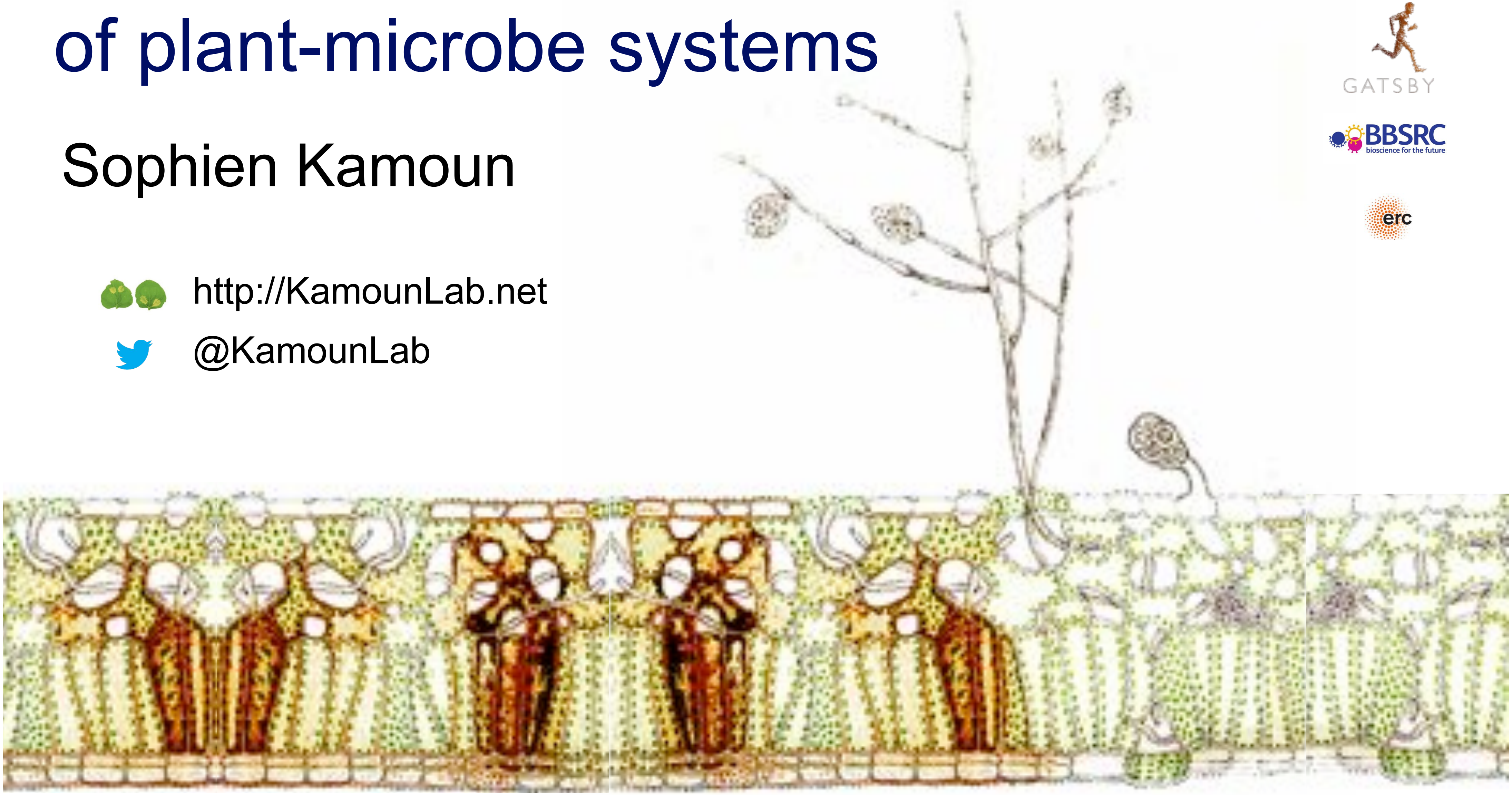
http://KamounLab.net

@KamounLab

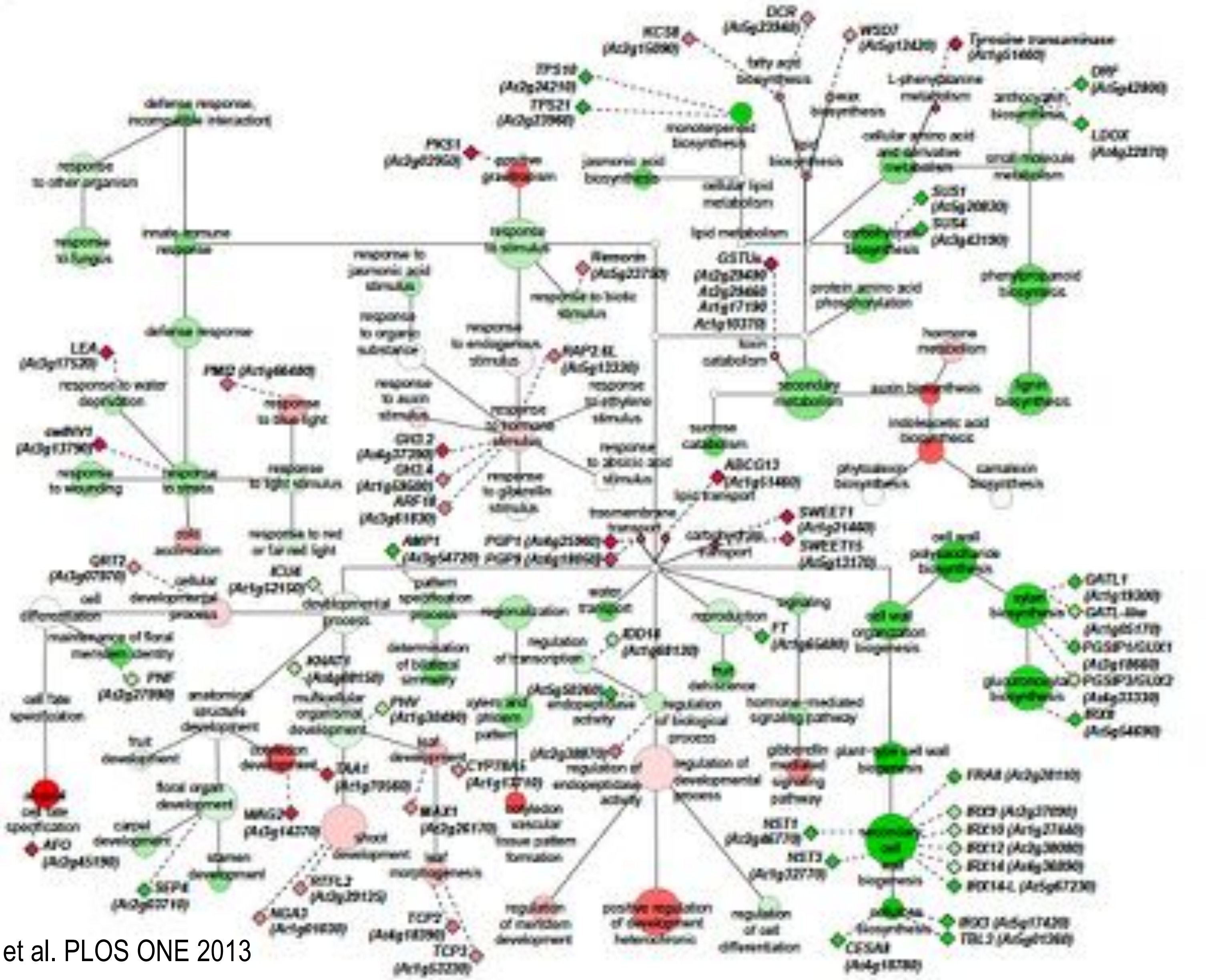
GATSBY

BBSRC bioscience for the future

erc

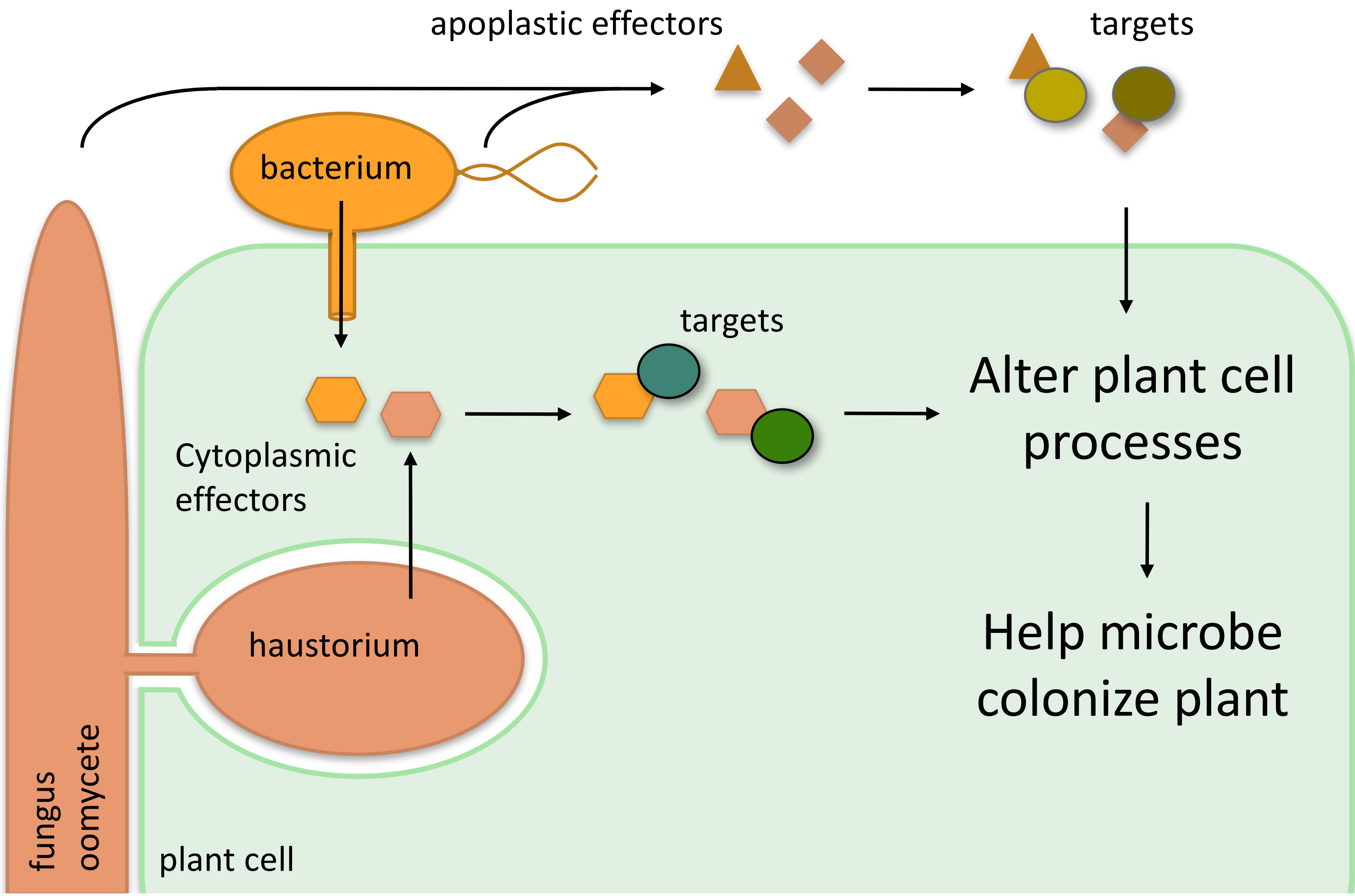


Flower mimicry by the rust fungus *Puccinia monoica*



Cano et al. PLOS ONE 2013

Microbes alter plant cell processes using both apoplastic and translocated effectors



Win, J., Chaparro-Garcia, A., Belhaj, K., Saunders, D.G.O., Yoshida, K., Dong, S., Schornack, S., Zipfel, C., Robatzek, S., Hogenhout, S.A., and Kamoun, S. Cold Spring Harbor Symposium on Quantitative Biology, 2013; Dodds, P.N., and Rathjen, J.P. Nature Reviews Genetics, 2010

Effectors - sensus Dawkins in “The extended phenotype: the long reach of the gene” p. 210, 1981

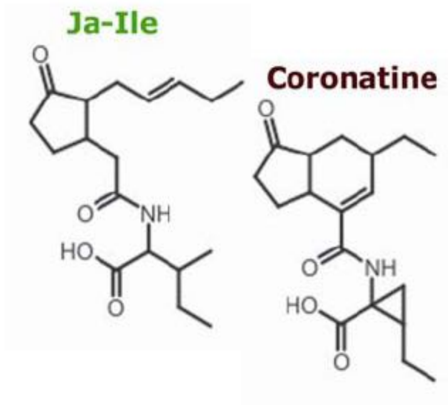
“...parasite genes having phenotypic expression in host bodies and behavior”

Principles of effector biology

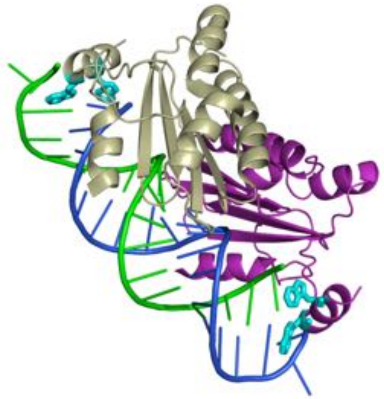
- Current paradigm – effector activities are key to understanding parasitism (and symbiosis)
- Secreted by a diversity of plant-associated organisms, e.g. bacteria, fungi, oomycetes, nematodes, insects
- Operationally, effectors are plant proteins and metabolites – encoded by genes in pathogen genomes but function in (inside) plant cells

Effectors are extraordinary examples of biological innovation

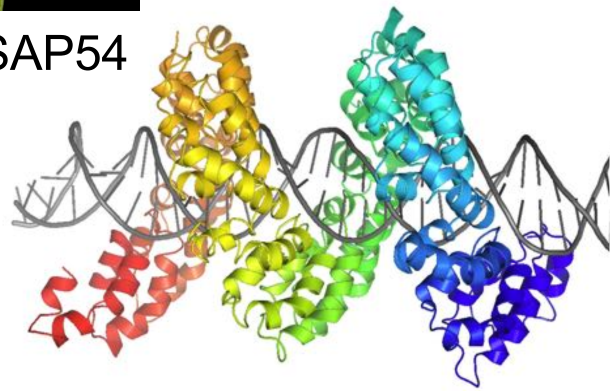
- *Some of most remarkable molecules known to function inside plant cells*
  - Hormones and hormone mimics
  - Modulators of basic plant processes, such as immunity and development
  - Virus suppressors of silencing
  - TALEs
  - etc.



P19-siRNA complex

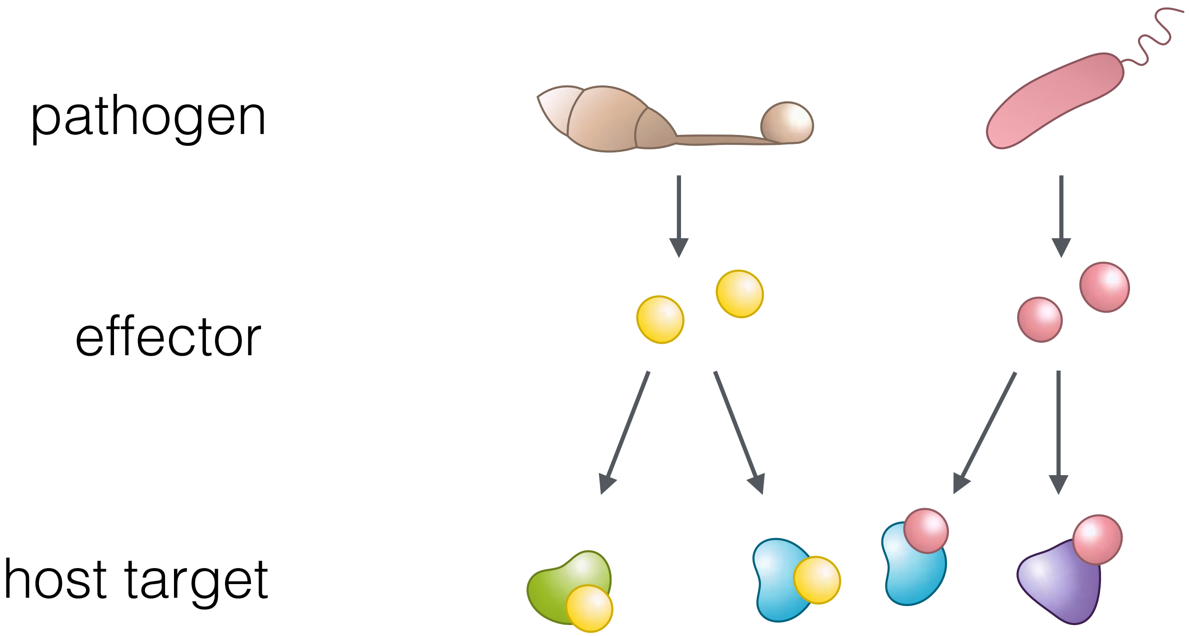


phytoplasma SAP54



TALE-DNA complex

Pathogens secrete effectors to alter plant cell processes and establish a susceptible state



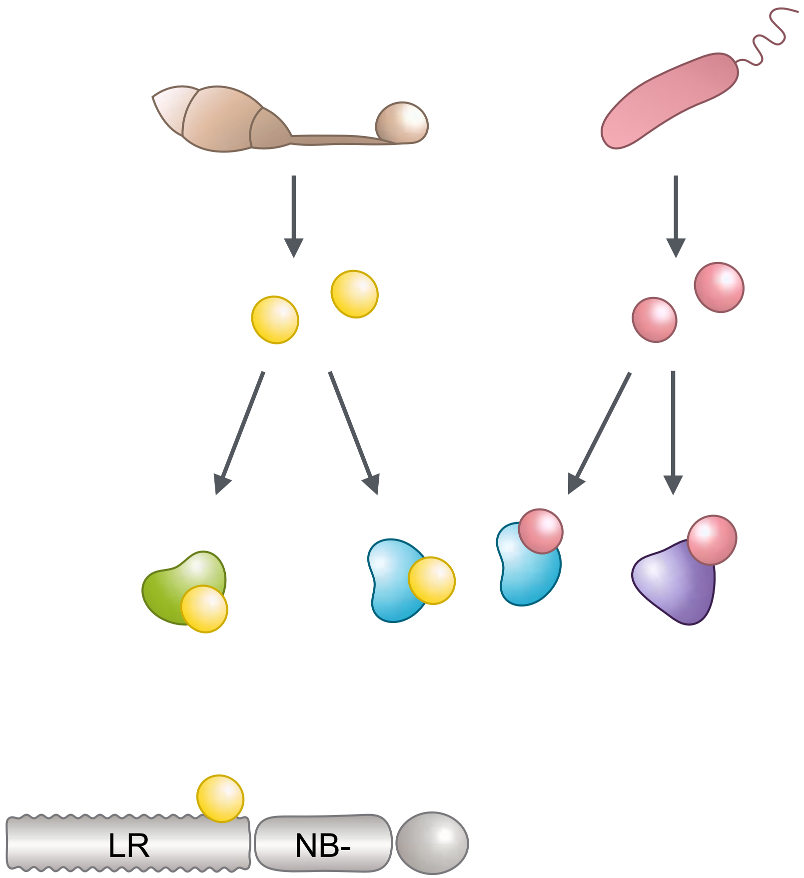
Some effectors "*trip the wire*" and activate immunity in specific plant genotypes

pathogen

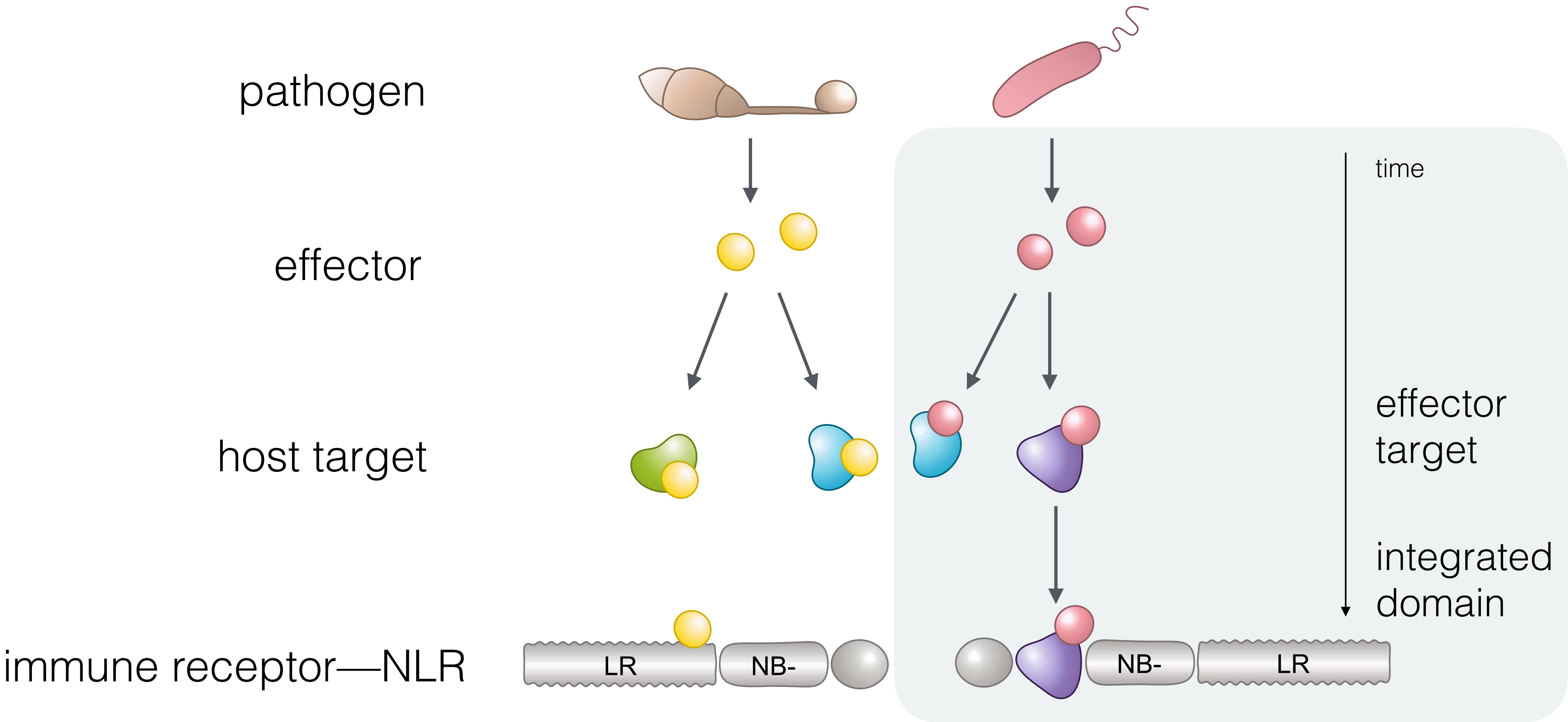
effector

host target

immune receptor—NLR

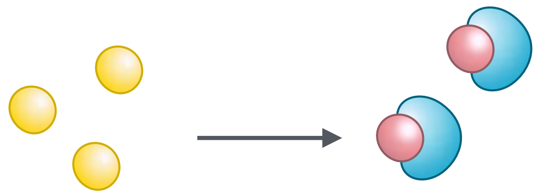


Integrated Domains — the merger of effector biology and NLR biology

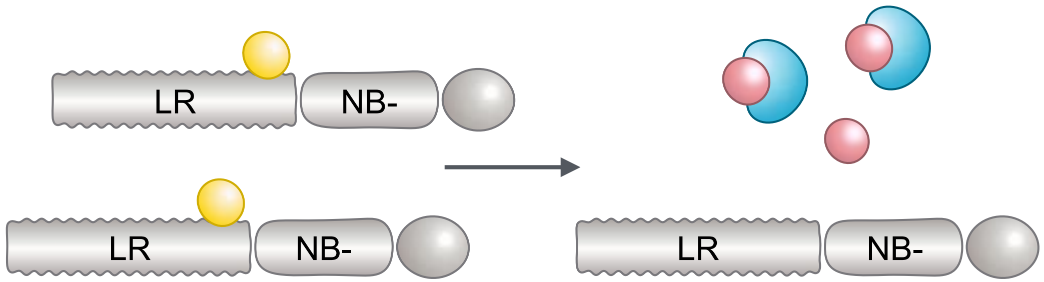


How do pathogen effectors evolve?

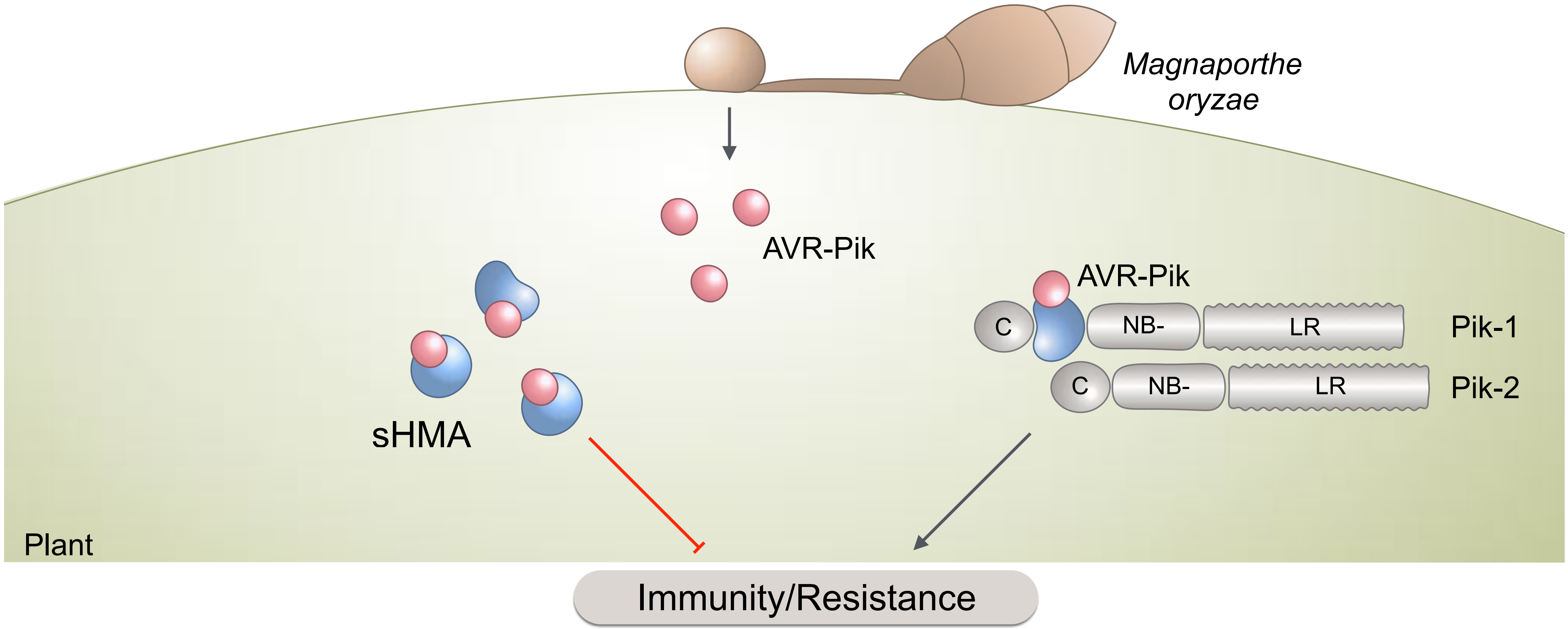
- Effectors adapt to (new) host targets, e.g. after a host jump



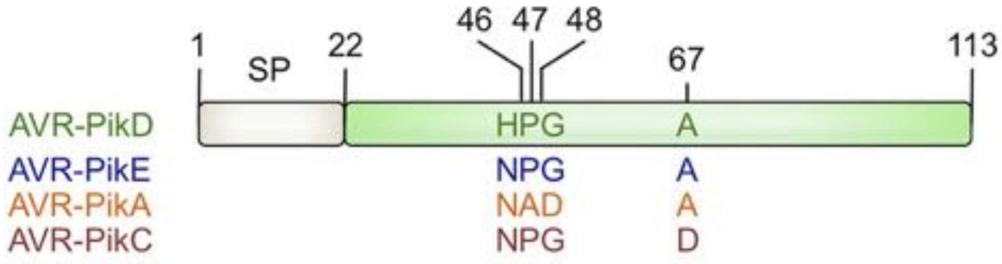
- Effectors evade immune receptors
    - stealthy mutants that retain virulence activity
    - pseudogenization, deletion, gene silencing

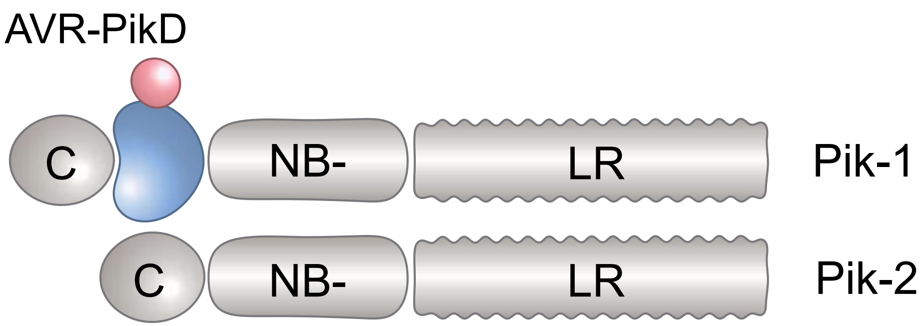


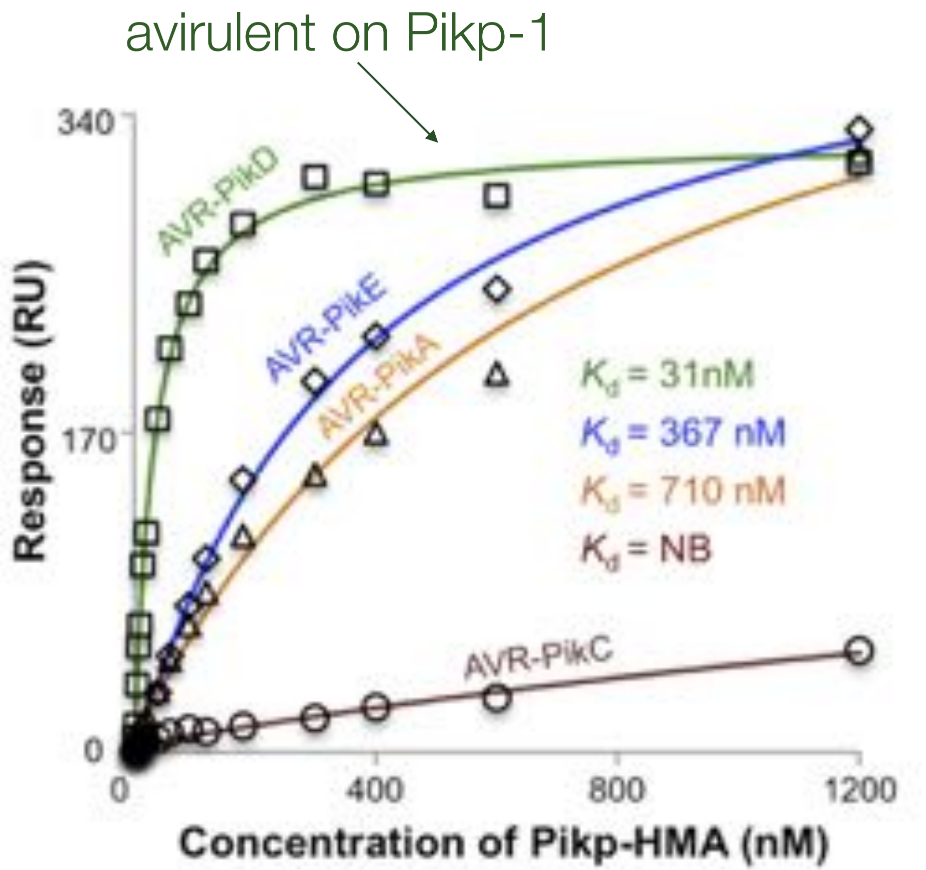
An effector target, HMA, integrated into Pik-1 NLR during rice evolution to bait the blast fungus effector AVR-Pik



Stealthy AVR-Pik variants evade activation of Pik-1 but retain capacity to bind HMA proteins

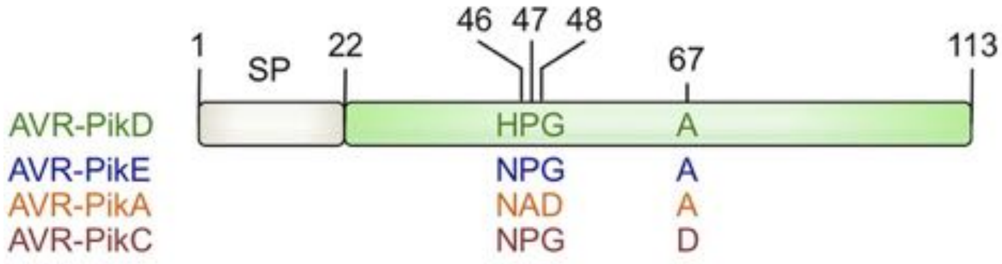


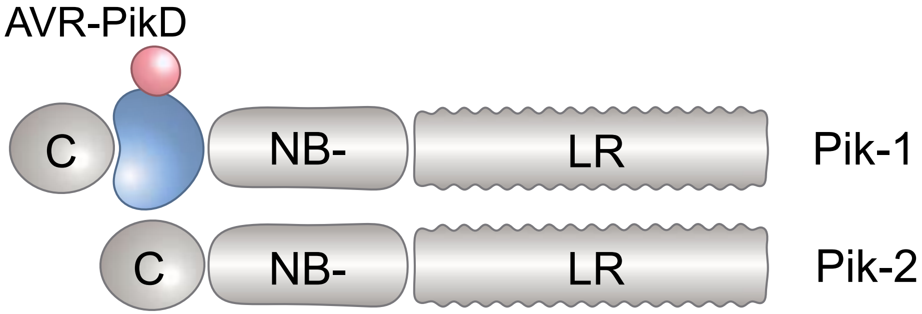


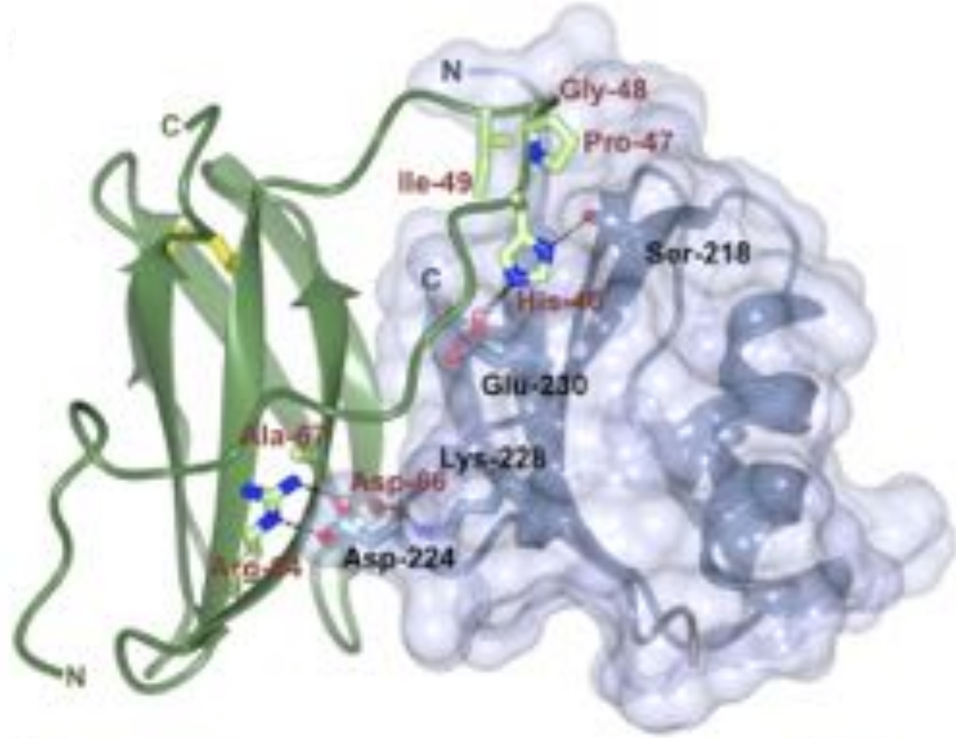


Maqbool *et al.* eLife 2015

Stealthy AVR-Pik variants evade activation of Pik-1 but retain capacity to bind HMA proteins

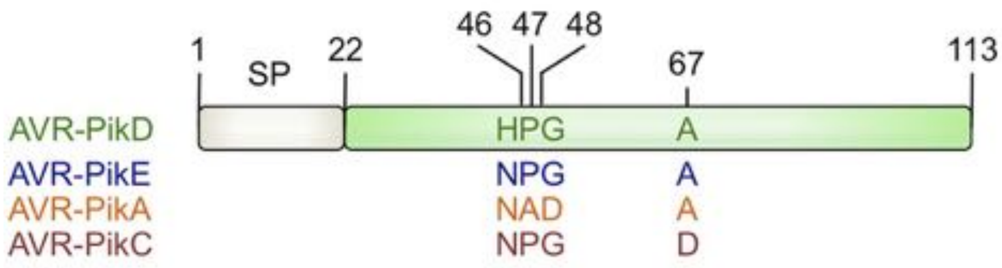


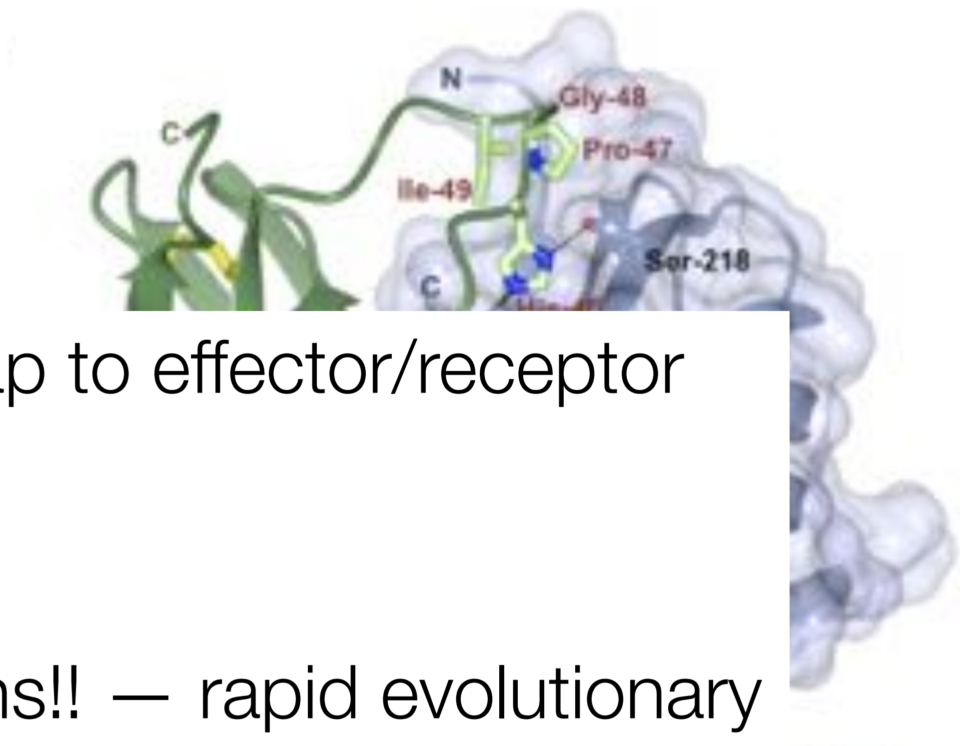




Maqbool *et al.* eLife 2015

Stealthy AVR-Pik variants evade activation of Pik-1 but retain capacity to bind HMA proteins



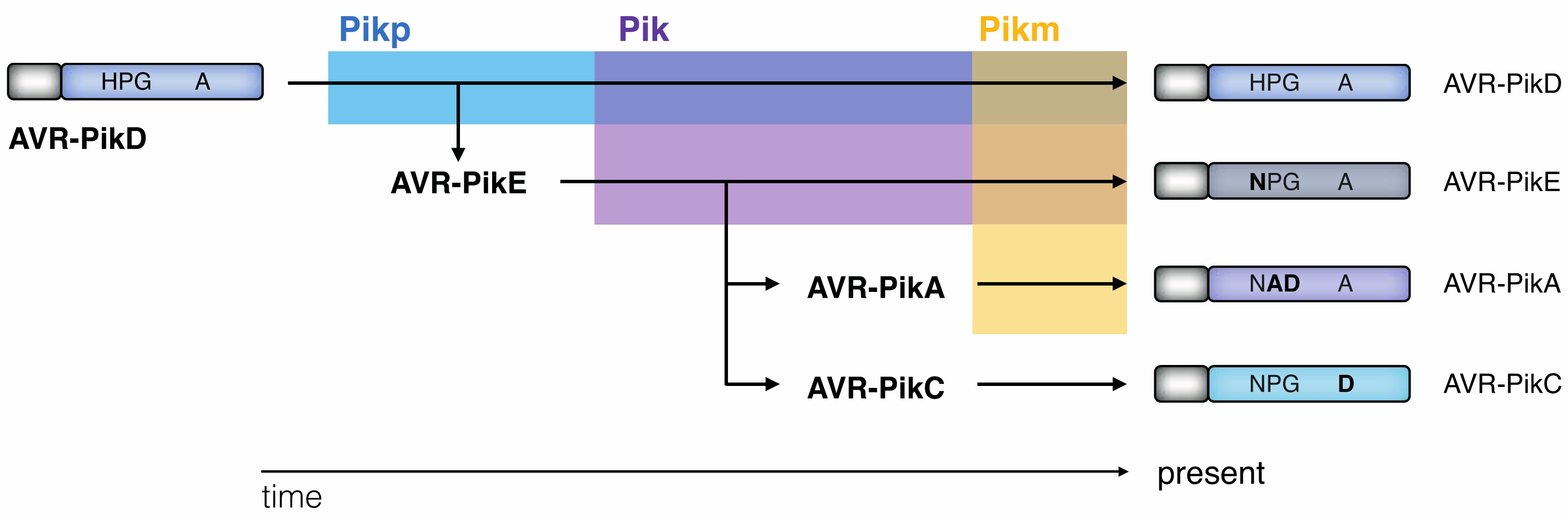


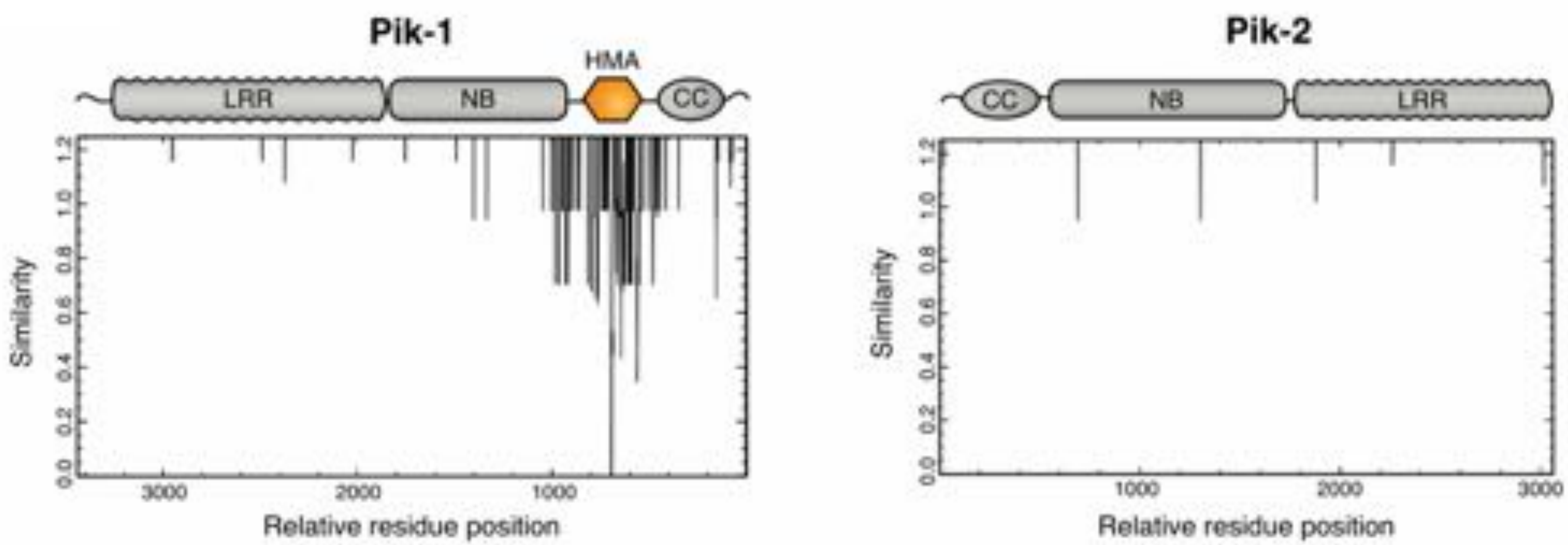
★ All amino acid mutations map to effector/receptor binding interface — adaptive!!

★ No synonymous substitutions!! — rapid evolutionary adaptations

Maqbool *et al.* eLife 2015

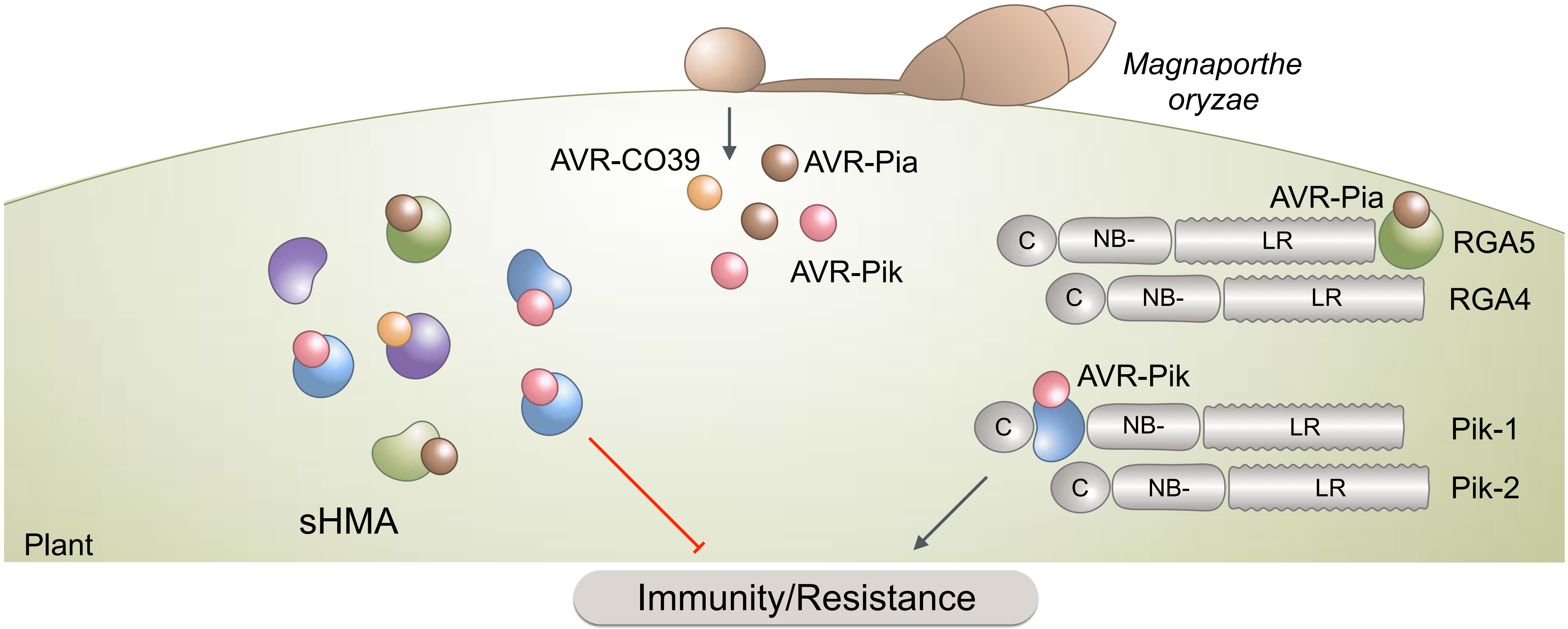
Magnaporthe oryzae AVR-Pik has evolved through an arms race with the rice immune receptor Pik



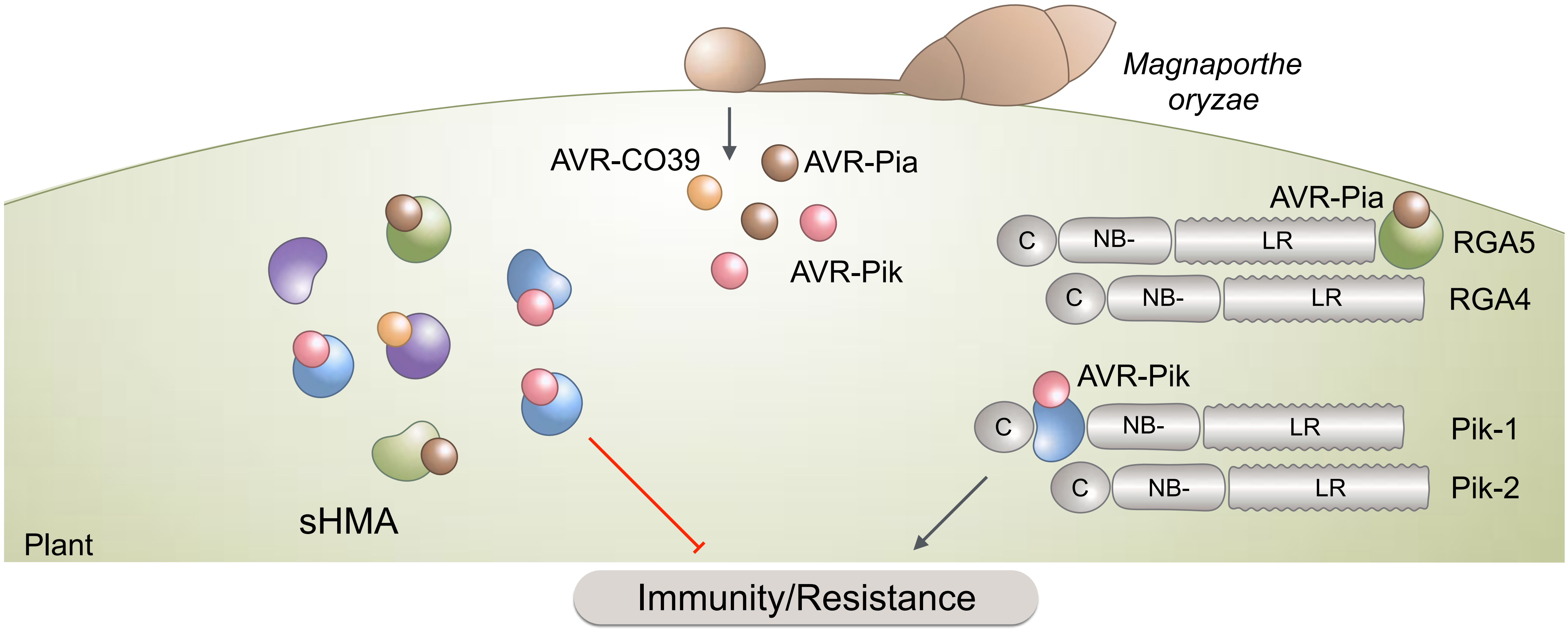


Bialas, Zess *et al.* bioRxiv 2017

Magnaporthe evolved multiple effectors to target host HMA proteins



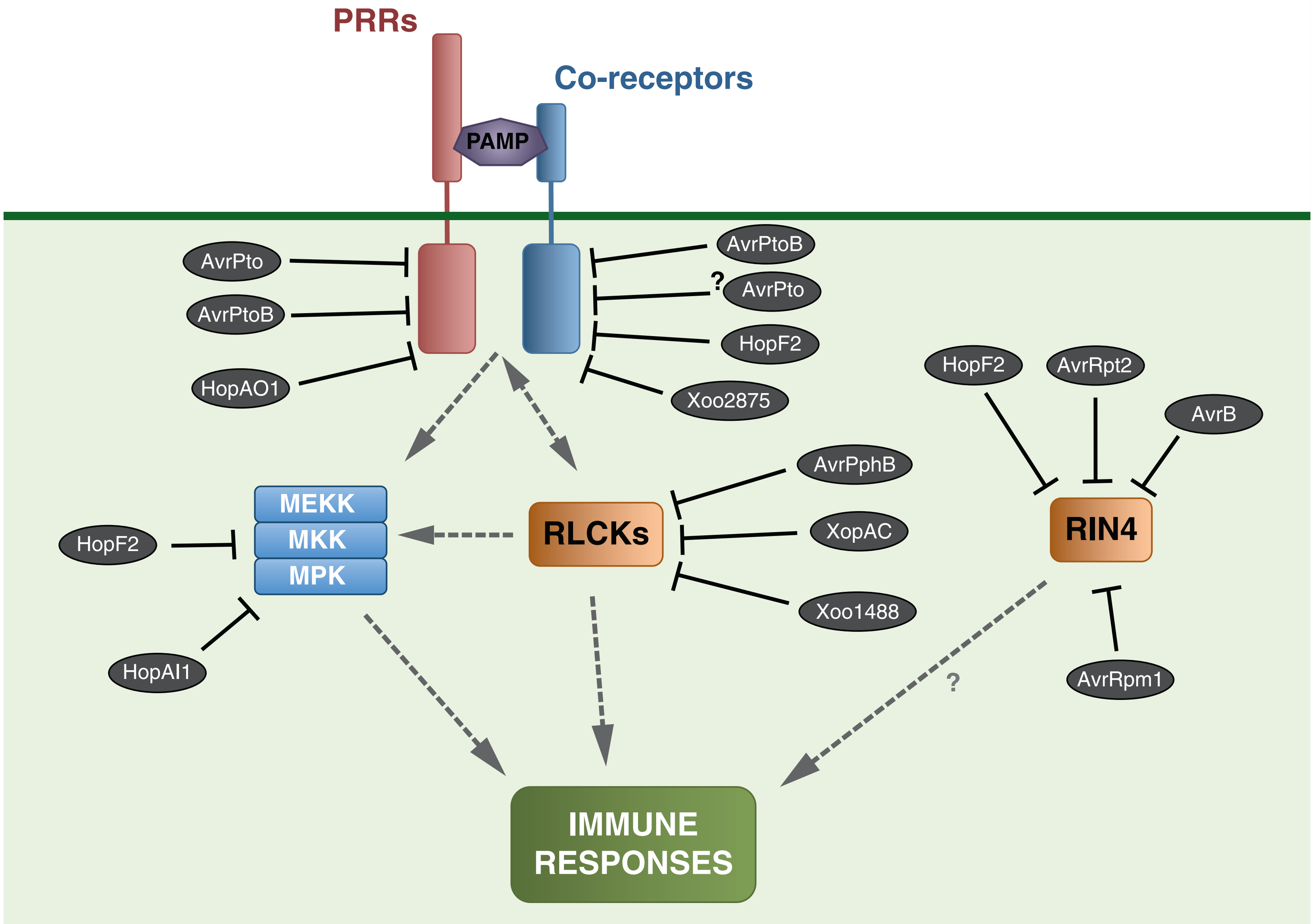
★Effector functional redundancy enables robustness and bet hedging…



ETP—Effector-targeted pathways: functional redundancy among pathogen effectors

- Effectors from a given pathogen tend to converge on particular host pathways
- Many effectors appear to be functionally redundant; act on different steps of an ETP or converge on same target (hub)
- Effectors from phylogenetically unrelated pathogens may converge on the same host targets

ETP—Effector-targeted pathways: functional redundancy among pathogen effectors



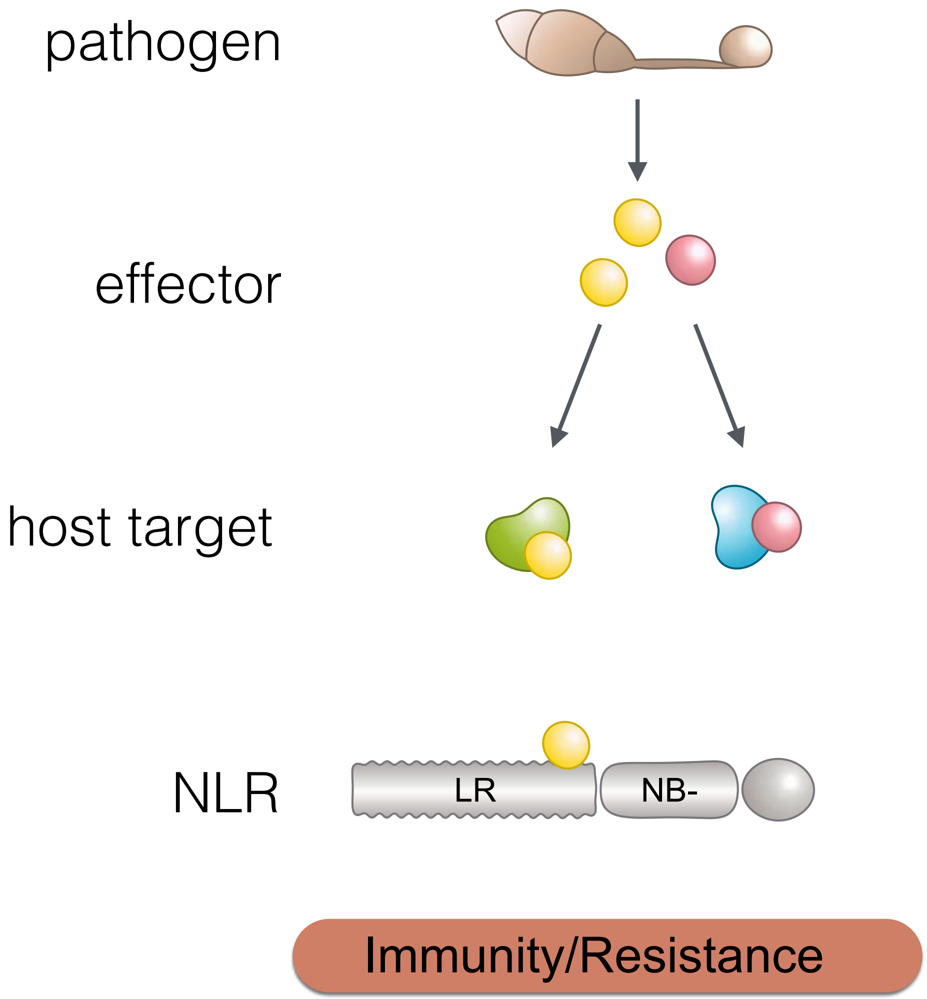
Macho and Zipfel
Curr Opin Microbiol 2015

Effectors from a given pathogen taxon tend to converge on particular host pathways

- Plant viruses suppress RNA silencing
- Bacterial type III effectors suppress plant immunity, particularly PAMP signalling pathways
- Fungal plant pathogen effectors disable chitin perception and chitin-elicited immunity
- etc.

★Define plant pathogens by the nature of the host processes they must impair?

NLR (NB-LRR) immune receptors can respond to effectors from biotrophs



Sophien Kamoun
@KamounLab

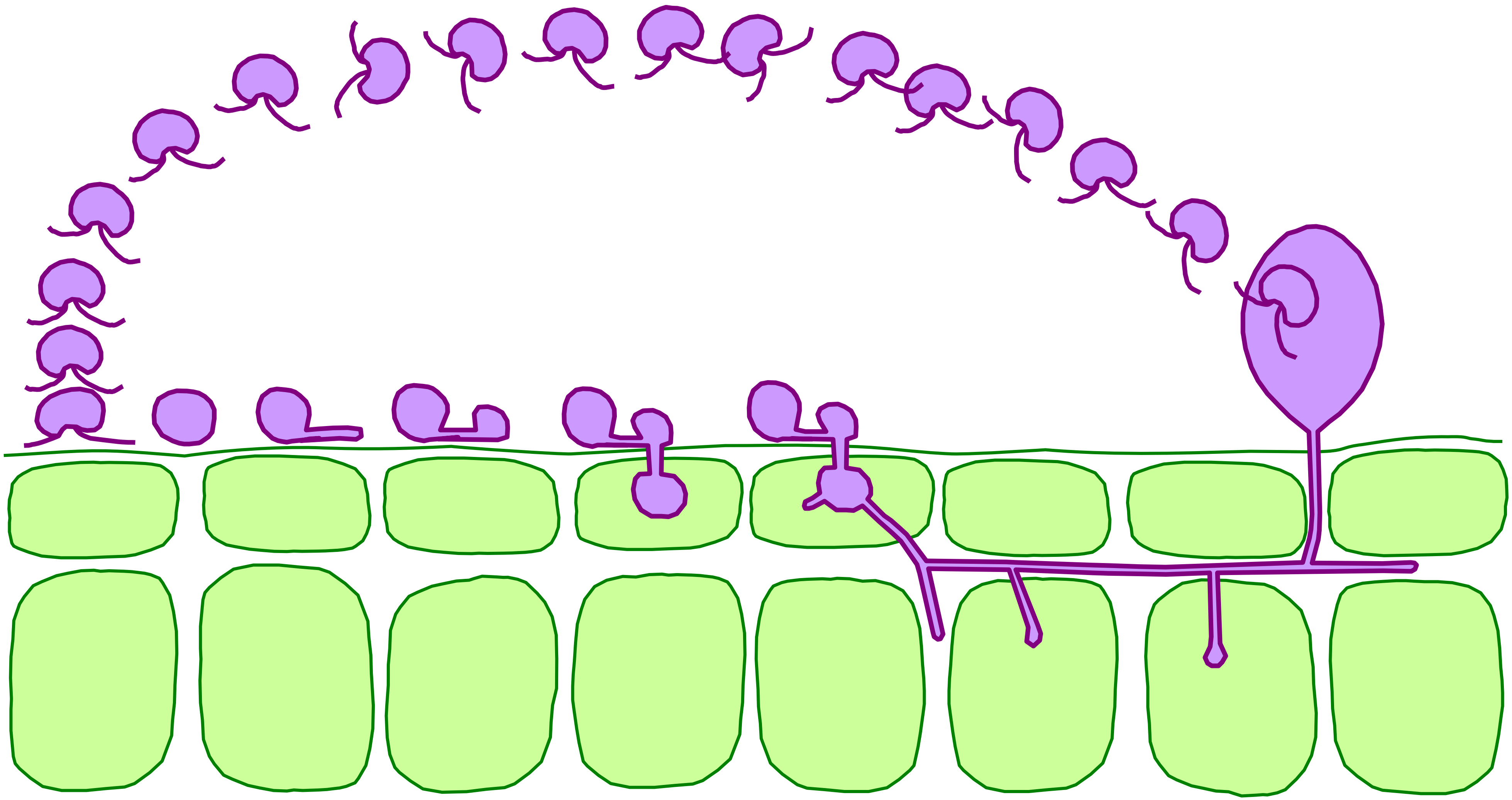
NB-LRRs that confer susceptibility should be called ARESISTANCE genes in line with AVIRULENCE terminology [just kiddin] #FGC11 Wolpert talk

12:40 AM - 19 Mar 2011

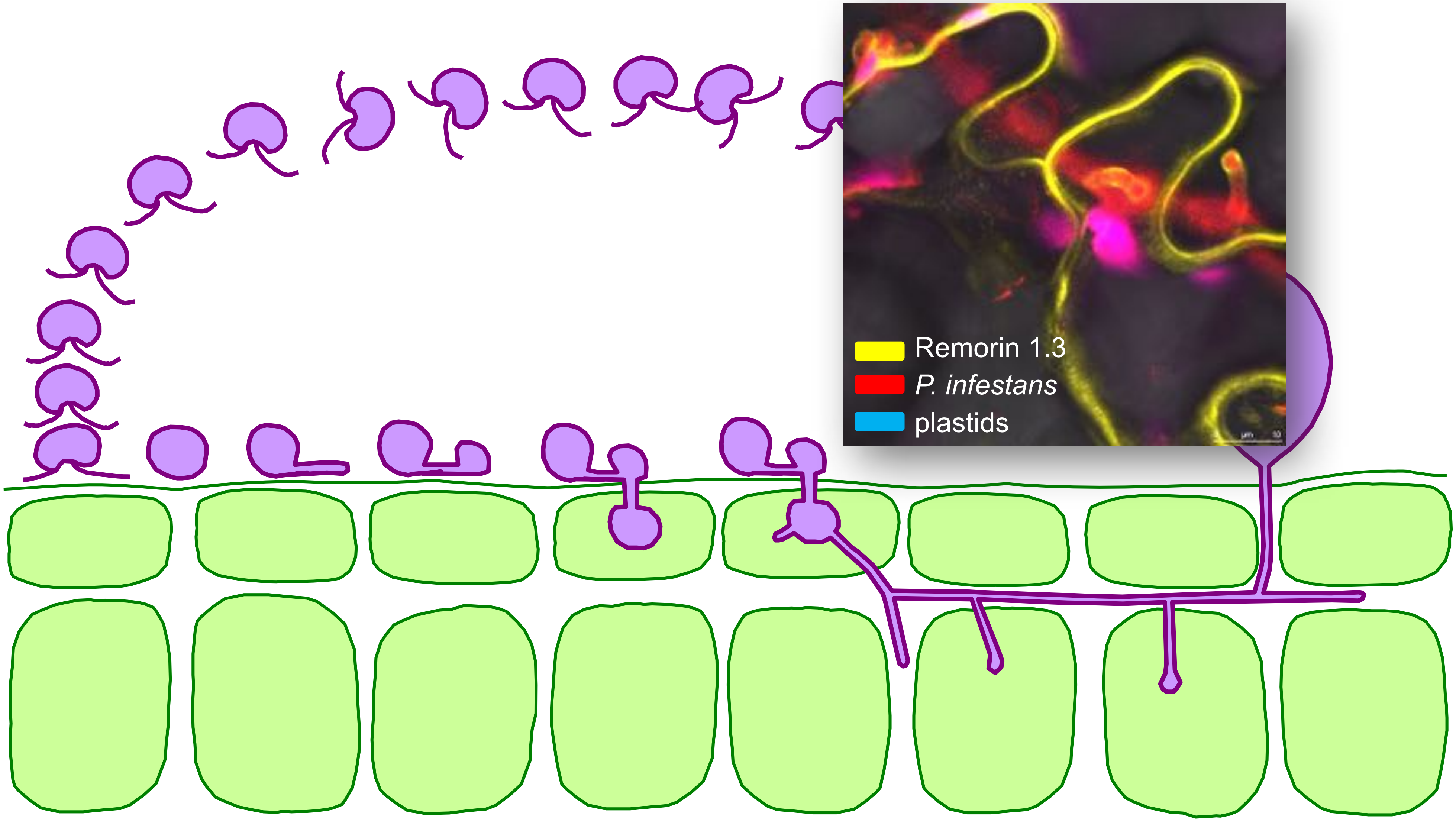
Phytophthora infestans

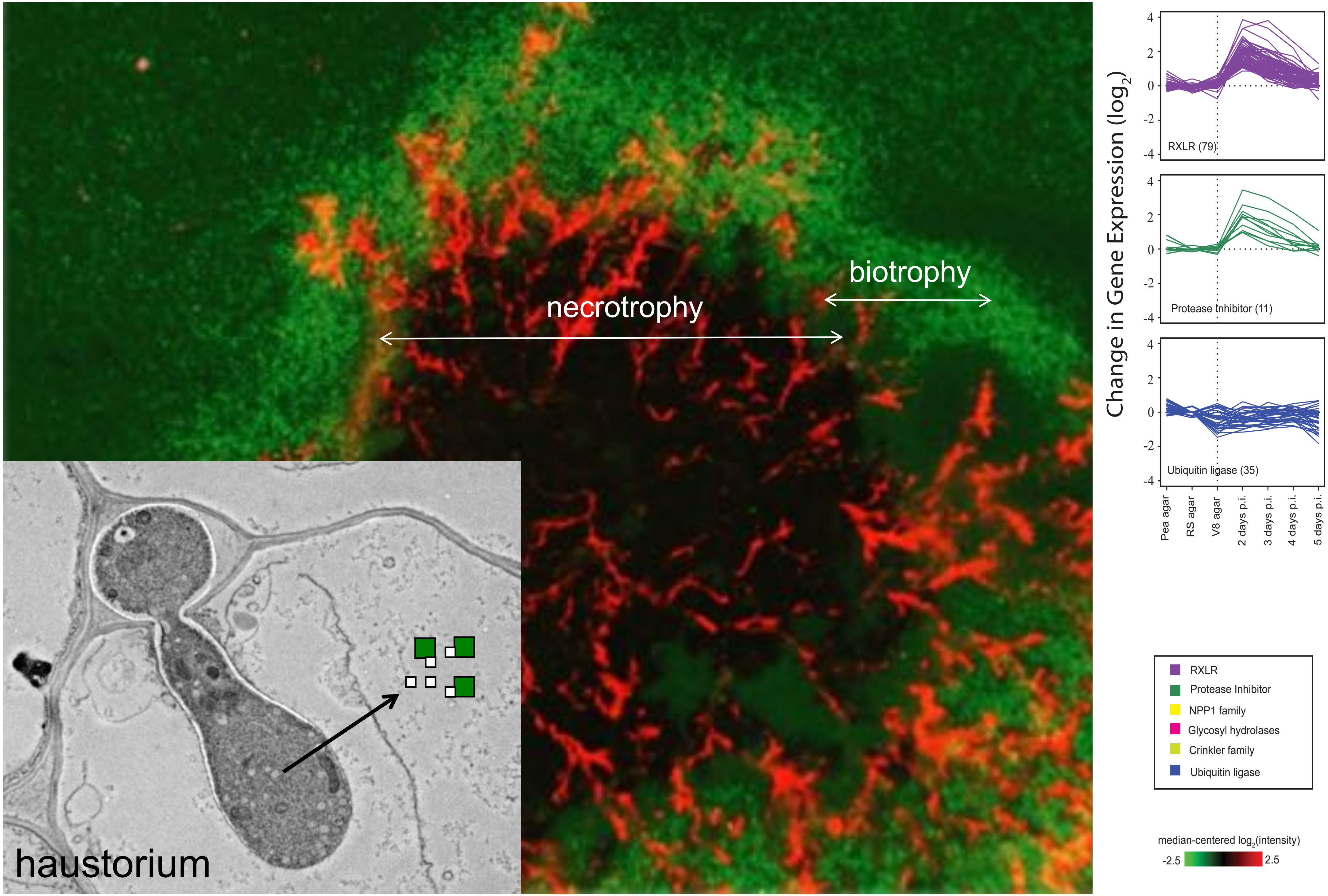
- fungus-like oomycete, hemibiotroph, haustoria
- potato and tomato late blight
- loads of effectors (hundreds)

The oomycete *Phytophthora infestans* – A *bona fide* extracellular pathogen of plants



The oomycete *Phytophthora infestans* – A *bona fide* extracellular pathogen of plants

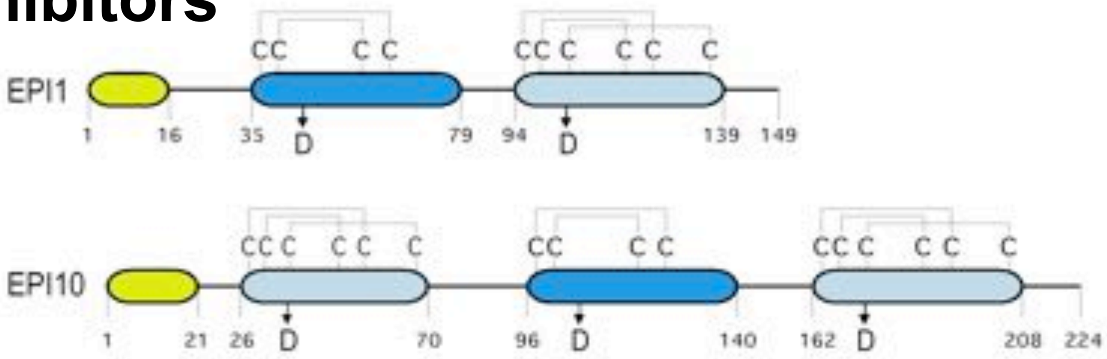




biotrophy
necrotrophy
haustorium
Change in Gene Expression ($\log_2$)
RXLR (79)
Protease Inhibitor (11)
Ubiquitin ligase (35)
4
2
0
-2
-4
Pea agar
RS agar
V8 agar
2 days p.i.
3 days p.i.
4 days p.i.
5 days p.i.
RXLR
Protease Inhibitor
NPP1 family
Glycosyl hydrolases
Crinkler family
Ubiquitin ligase
median-centered $\log_2$(intensity)
-2.5
2.5

The diverse effectors of *Phytophthora infestans*

Protease inhibitors

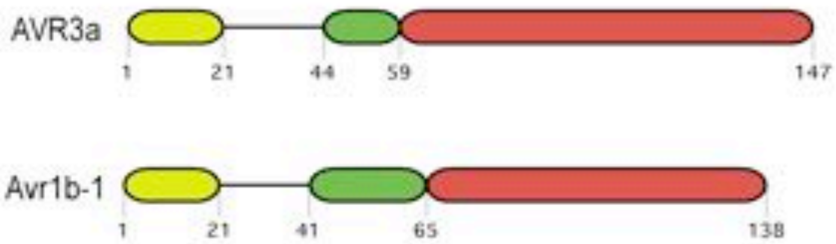


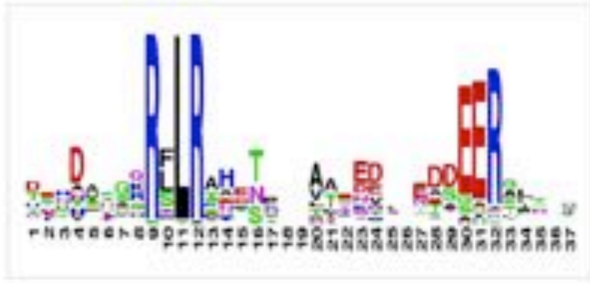
~38

Apoplastic

RXLR

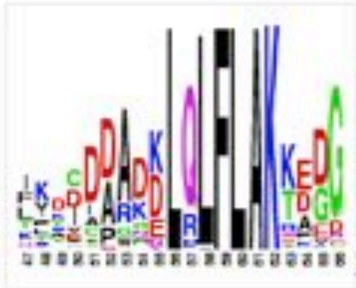
Host-translocated





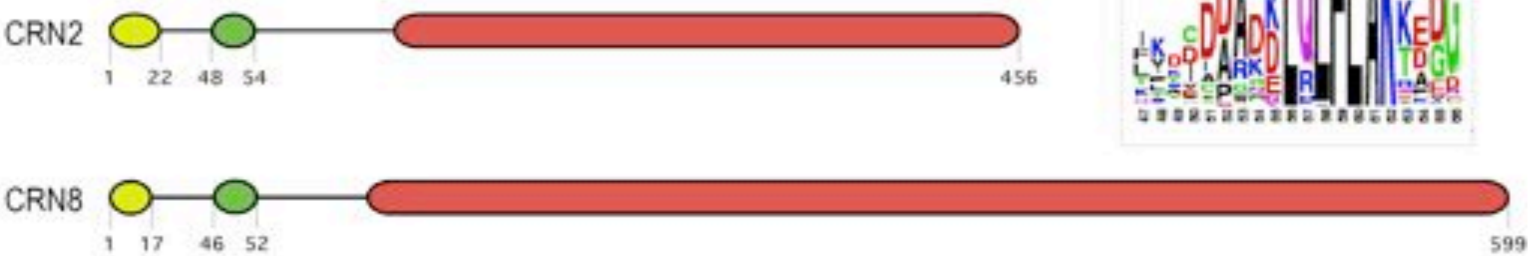
~550

Crinklers



~200

~250⊖



#EffectorWisdom /via Twitter

Kamoun Lab @ TSL
@KamounLab

Studying effector function is by definition a leap into the unknown. No clue where they would lead you. But you know it will be important...

Reply Delete Favorite

4 RETWEETS

8:16 AM - 6 Jun 12 via Twitter for iPhone · Embed

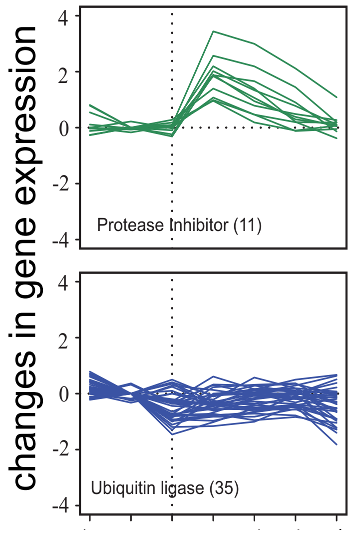
...otherwise no way they would evolve. Effectors result from an awesome genetic screen that took place in nature over millions of years

Reply Delete Favorite

4 RETWEETS

8:17 AM - 6 Jun 12 via Twitter for iPhone · Embed this Tweet

Kazal- and cystatin-like protease inhibitors – Apoplastic effectors of *Phytophthora*



2007

THE JOURNAL OF BIOLOGICAL CHEMISTRY

2004

A Kazal-like Extracellular Serine Protease Inhibitor from *Phytophthora infestans* Targets the Tomato Pathogenesis-related Protease P69B*

Received for publication, January 28, 2004, and in revised form, April 15, 2004
Published, JBC Papers in Press, April 19, 2004, DOI 10.1074/jbc.M400941200

Miaoying Tian, Edgar Huitema, Luis da Cunha, Trudy Torto-Alalibo, and Sophien Kamoun‡

From the Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

A *Phytophthora infestans* Cystatin-Like Protein Targets a Novel Tomato Papain-Like Apoplastic Protease[1[W][OA]]

Miaoying Tian[3], Joe Win[2], Jing Song[2], Renier van der Hoorn, Esther van der Knaap, and Sophien Kamoun*

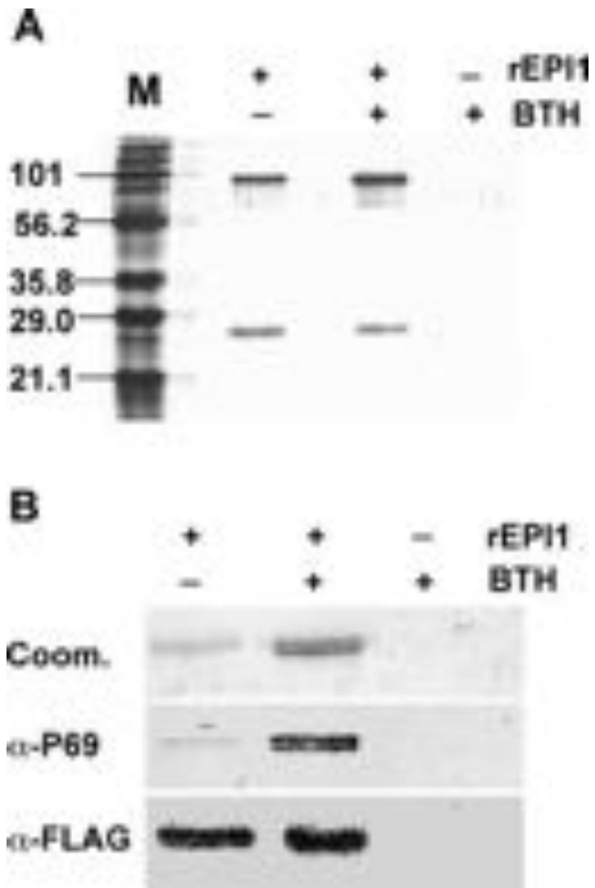
Department of Plant Pathology (M.T., J.W., J.S., S.K.) and Department of Horticulture and Crop Sciences (E.v.d.K.), The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691; and Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany (R.v.d.H.)

Apoplastic effectors secreted by two unrelated eukaryotic plant pathogens target the tomato defense protease Rcr3

2009

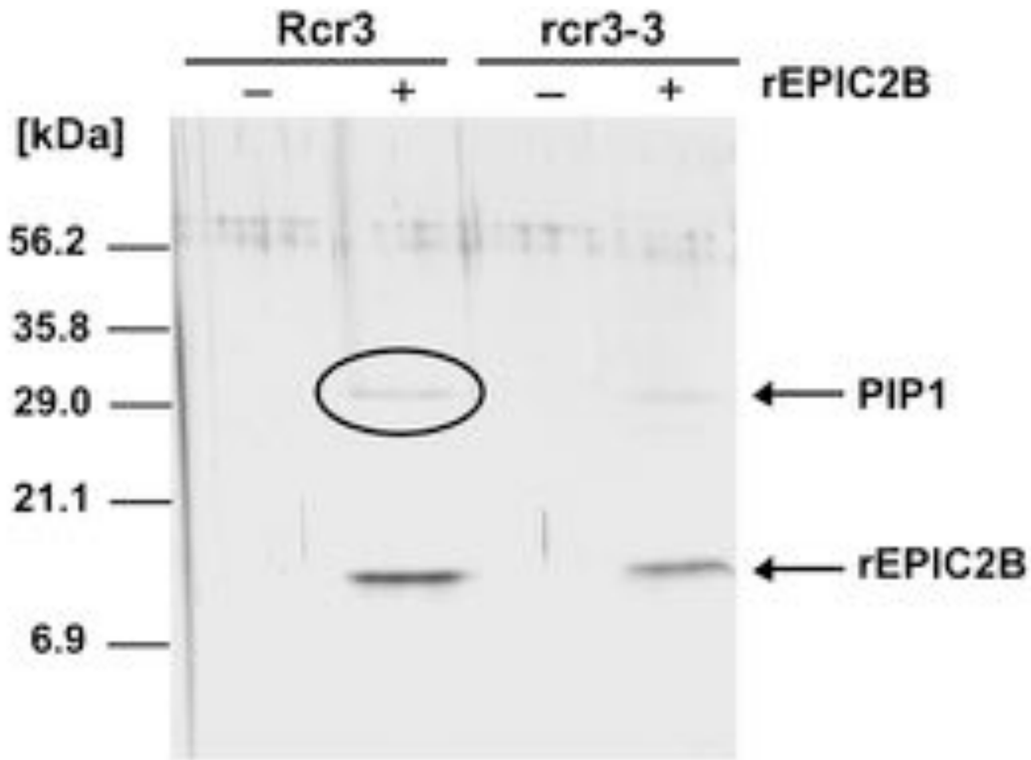
Jing Song[a], Joe Win[a,b], Miaoying Tian[a,1], Sebastian Schornack[b], Farnusch Kaschani[c], Muhammad Ilyas[c], Renier A. L. van der Hoorn[c], and Sophien Kamoun[a,b,2]

Identification of host targets of Kazal- and cystatin-like effectors by coimmunoprecipitation



Subtilisin-like Ser protease P69B

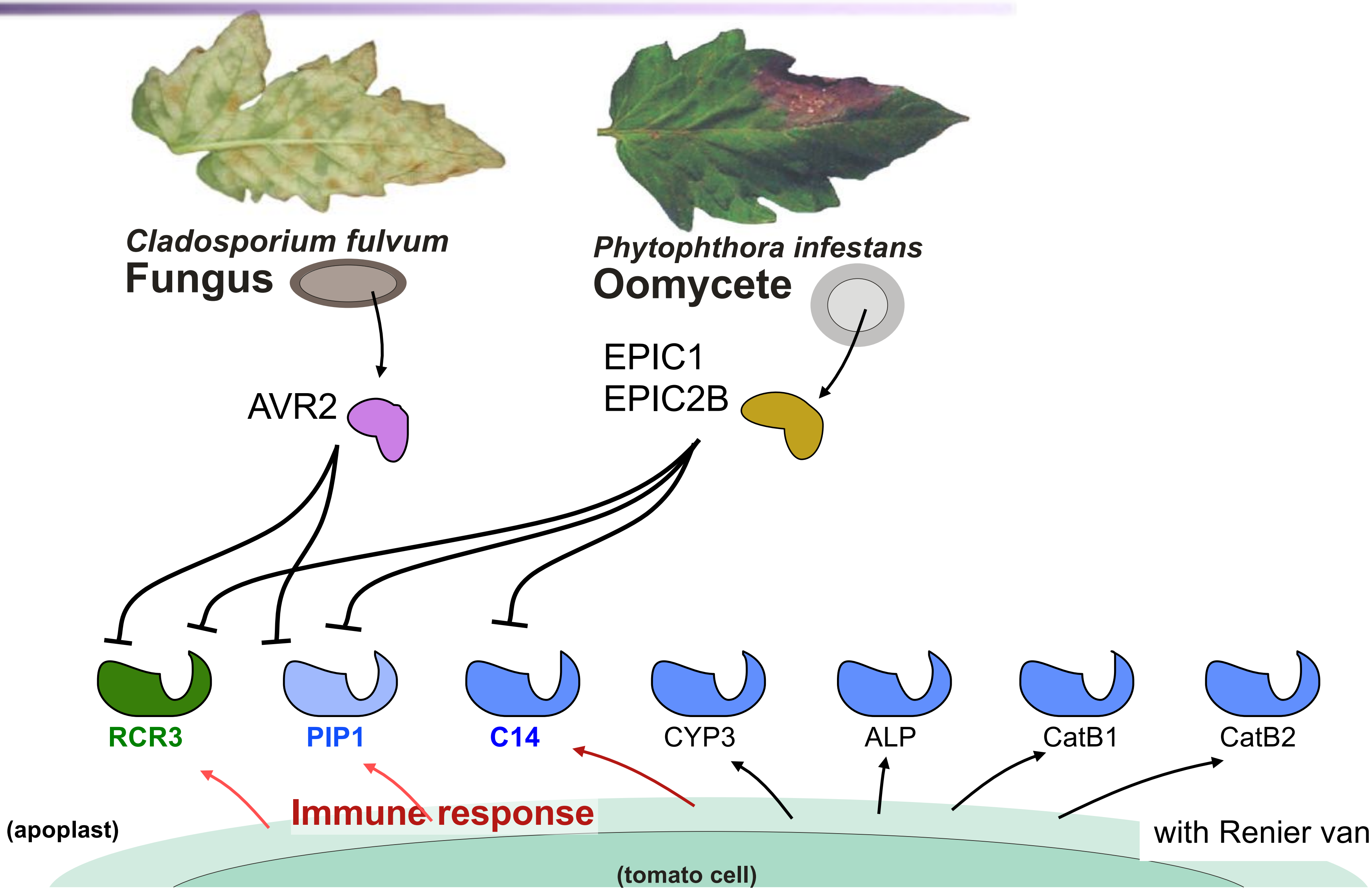
Tian *et al*. JBC (2004)



Papain-like Cys protease PIP1

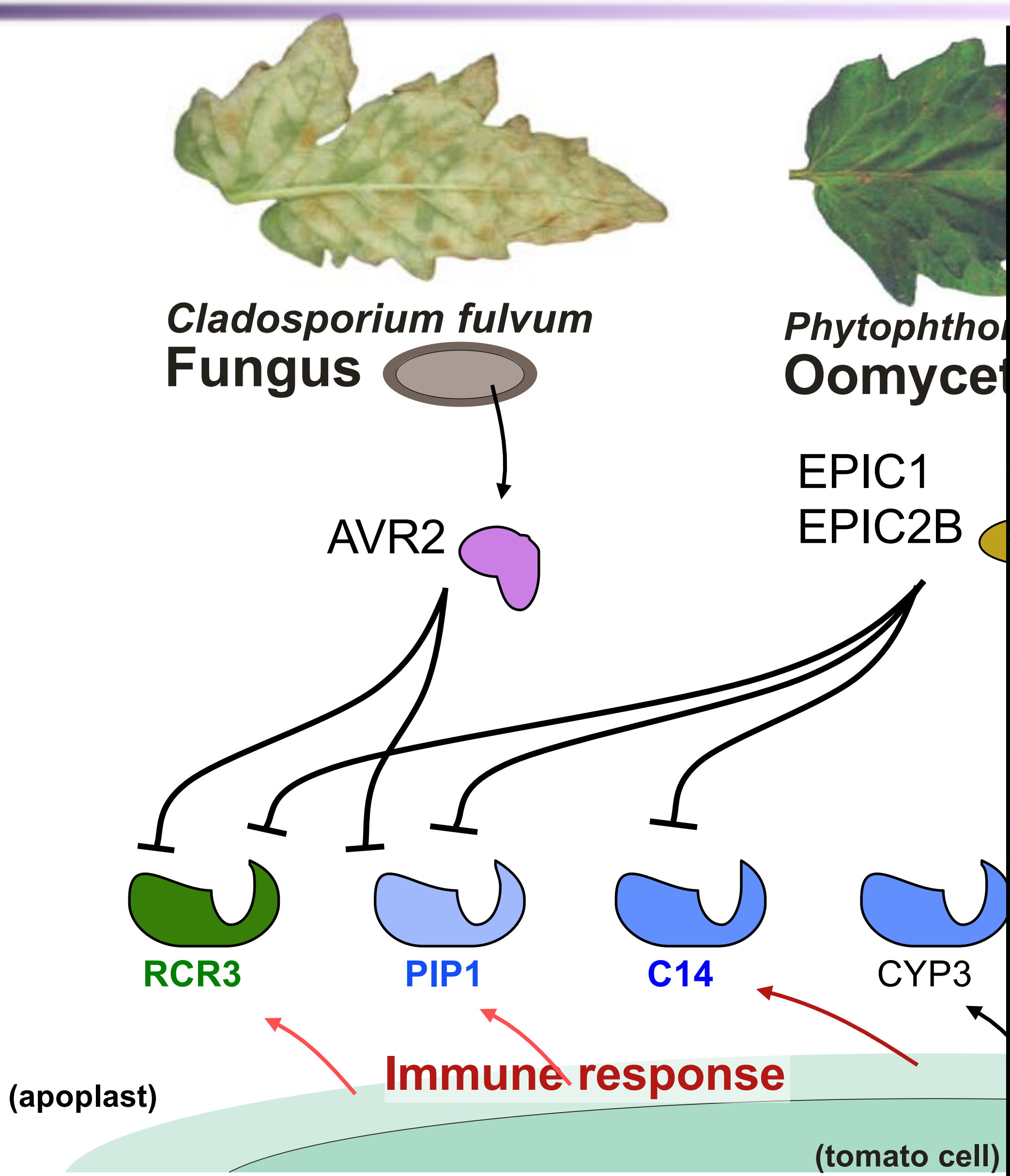
Tian *et al*. Plant Phys (2007)

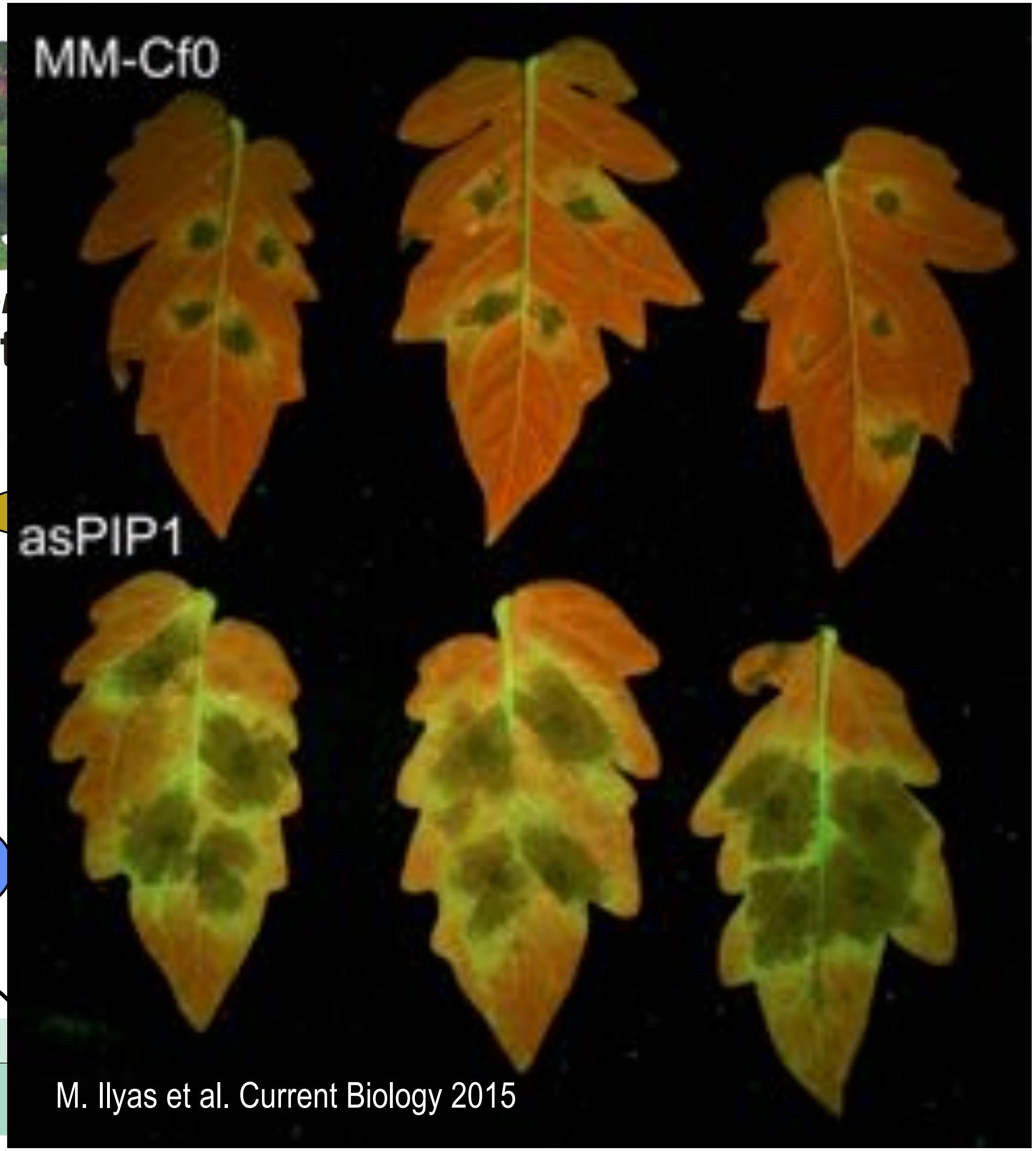
Filamentous pathogen inhibitor effectors revealed plant immune Cys proteases



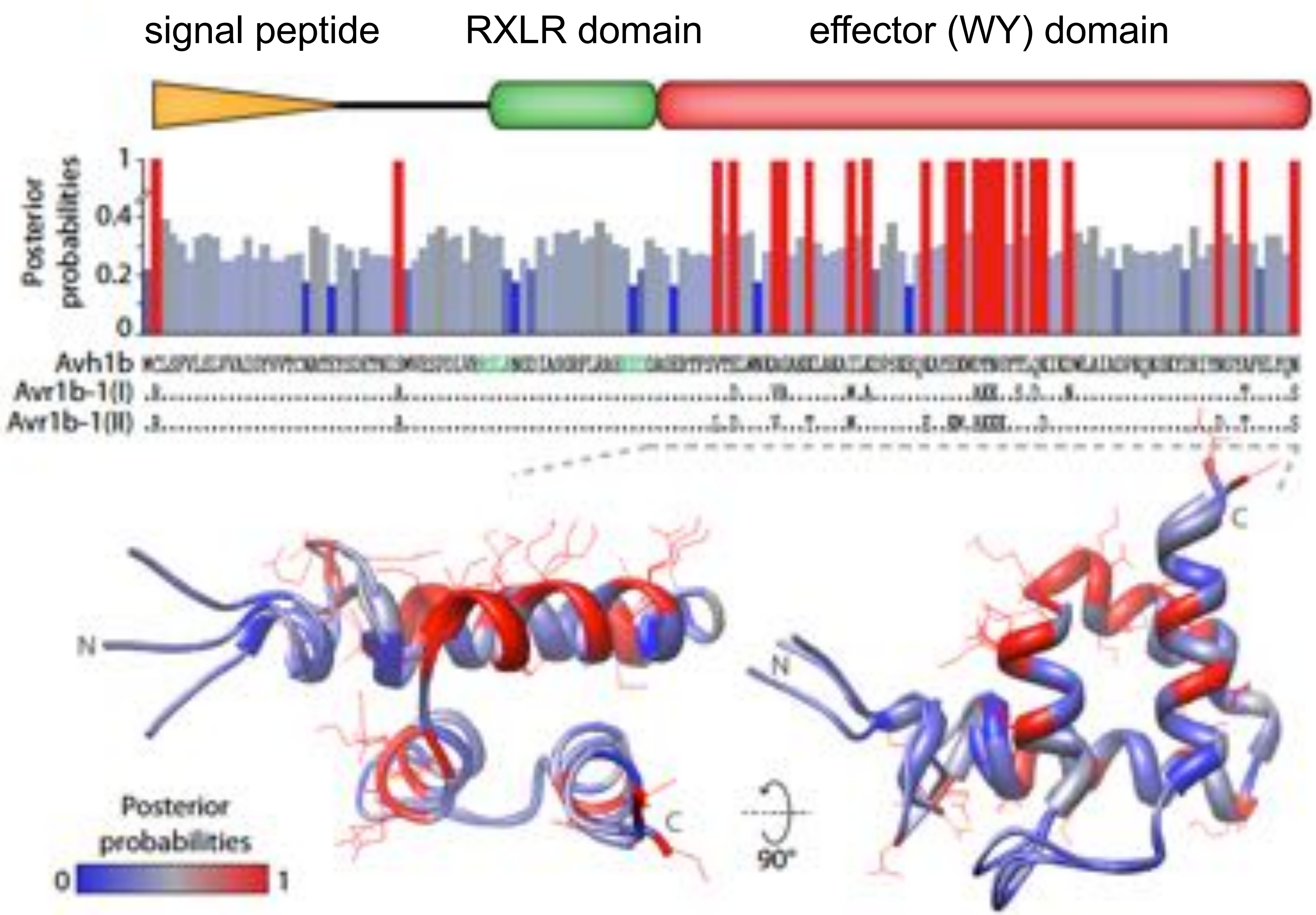
with Renier van der Hoorn

Filamentous pathogen inhibitor effectors revealed plant immune Cys proteases

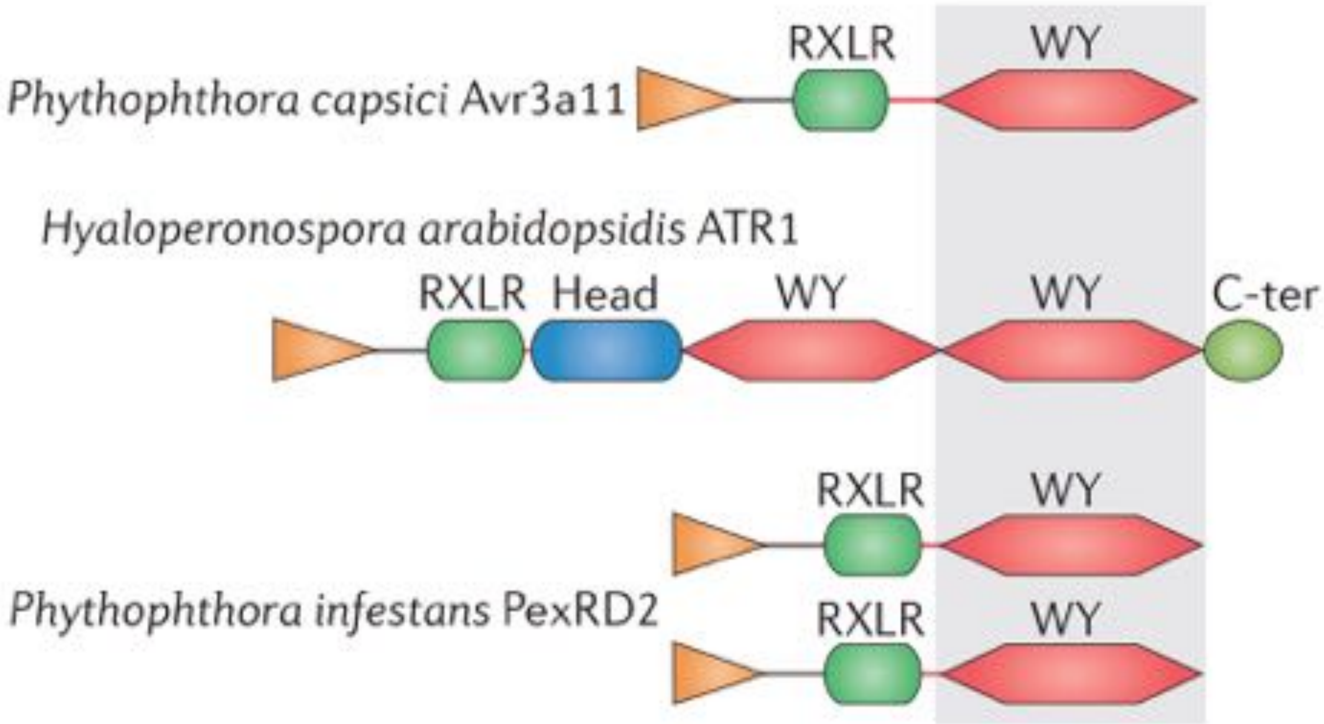


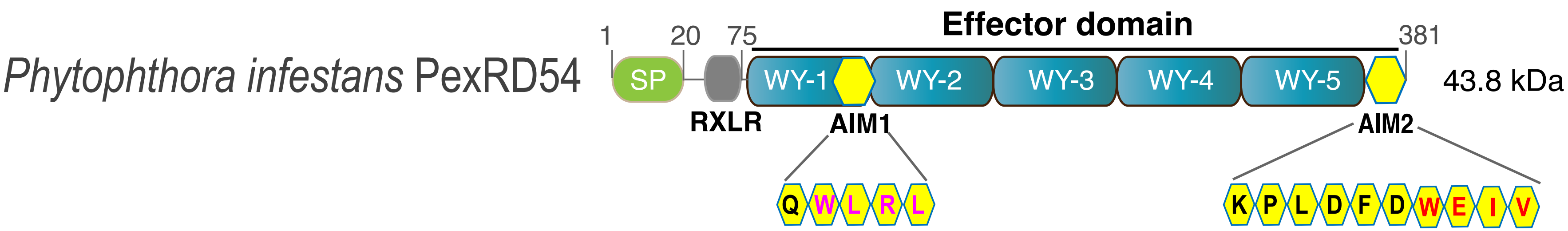


RXLR effectors are rapidly evolving modular proteins



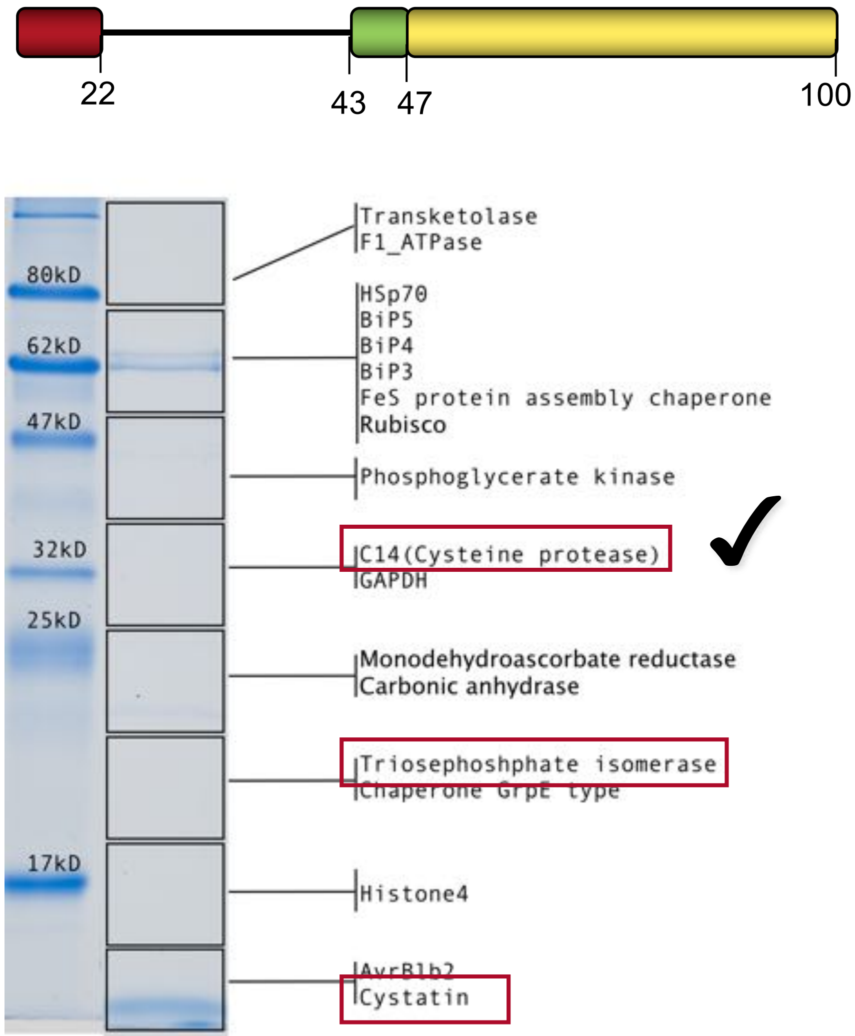
RXLR effectors can contain multiple variations of the WY domains, e.g. tandem duplications



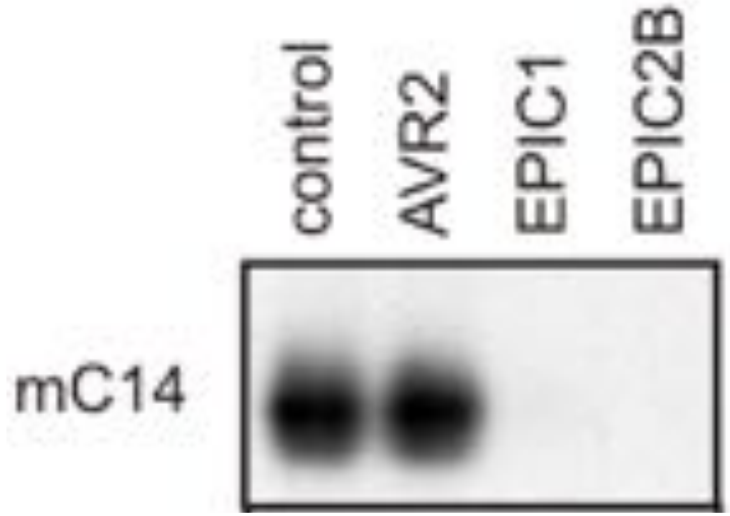


What are the host targets of AVRblb2?

Co-IP with FLAG-AVRblb2



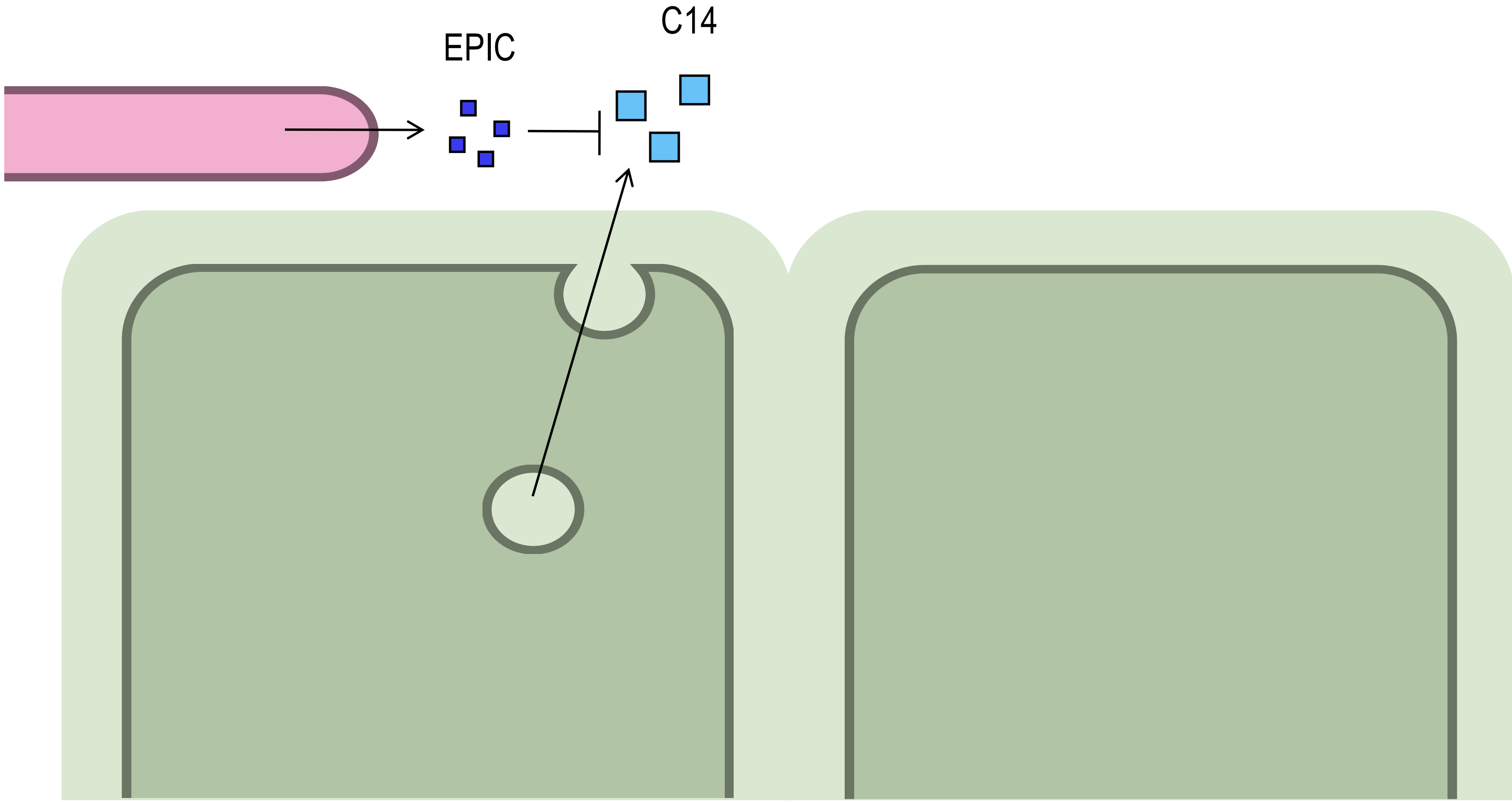
Tolga Bozkurt, Joe Win, Alex Jones



DCG04 protease activity assay

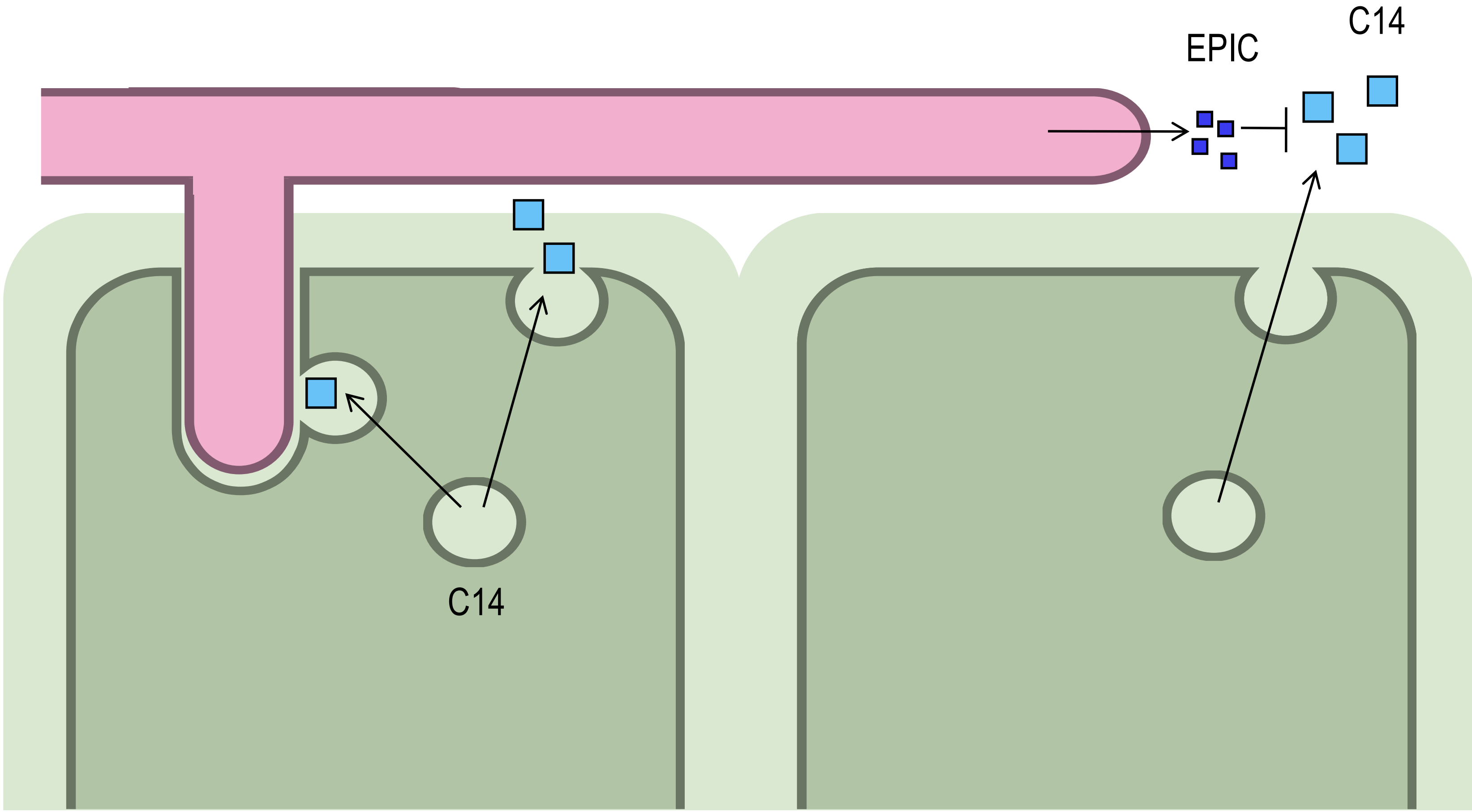
P. infestans EPICs inhibit C14 in the apoplast (M. Shabab and Renier van der Hoorn, Max Planck Inst. Cologne)

P. infestans evolved distinct effectors and mechanisms to interfere with apoplastic proteases



Kamoun & van der Hoorn Labs

P. infestans evolved distinct effectors and mechanisms to interfere with apoplastic proteases



Kamoun & van der Hoorn Labs

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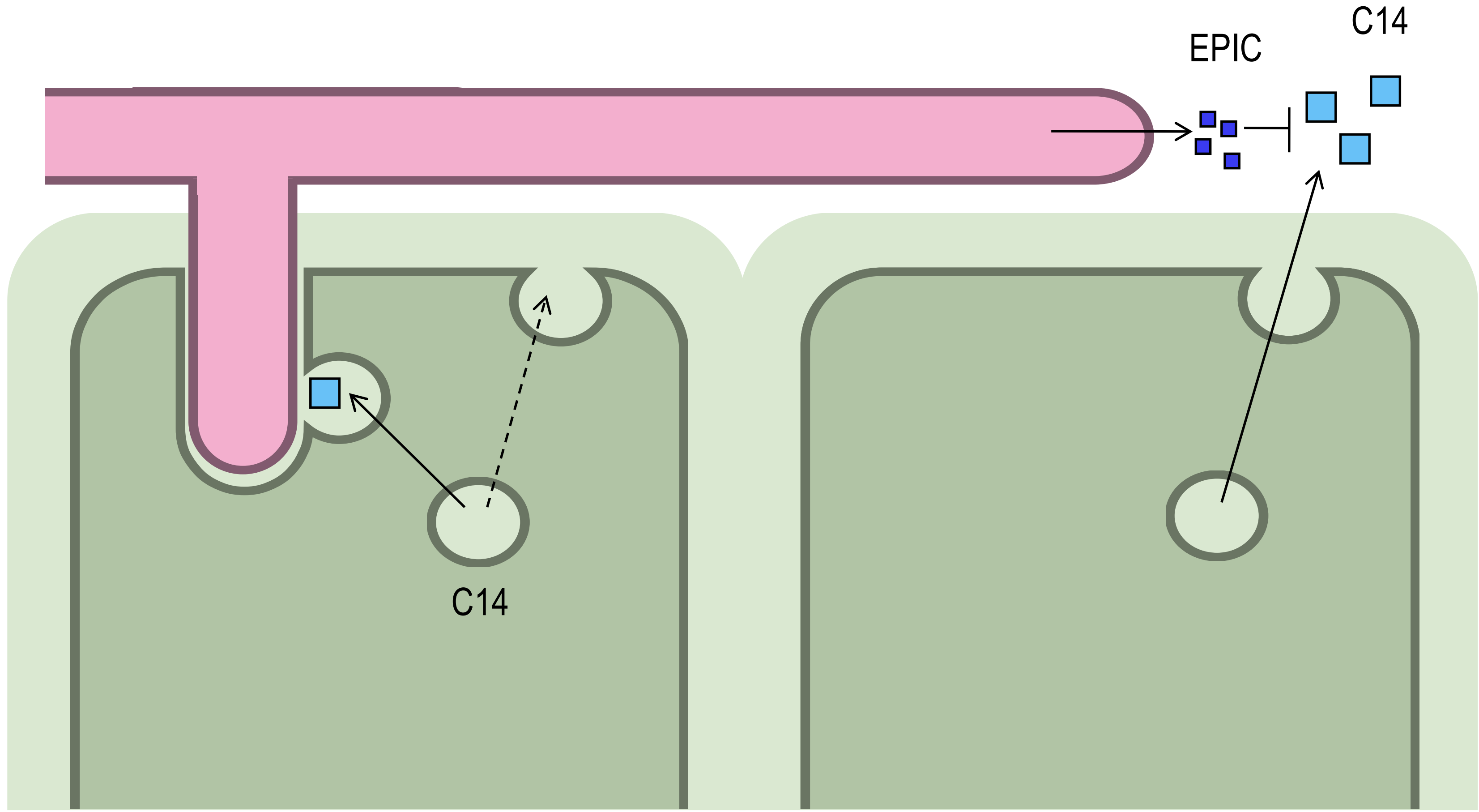
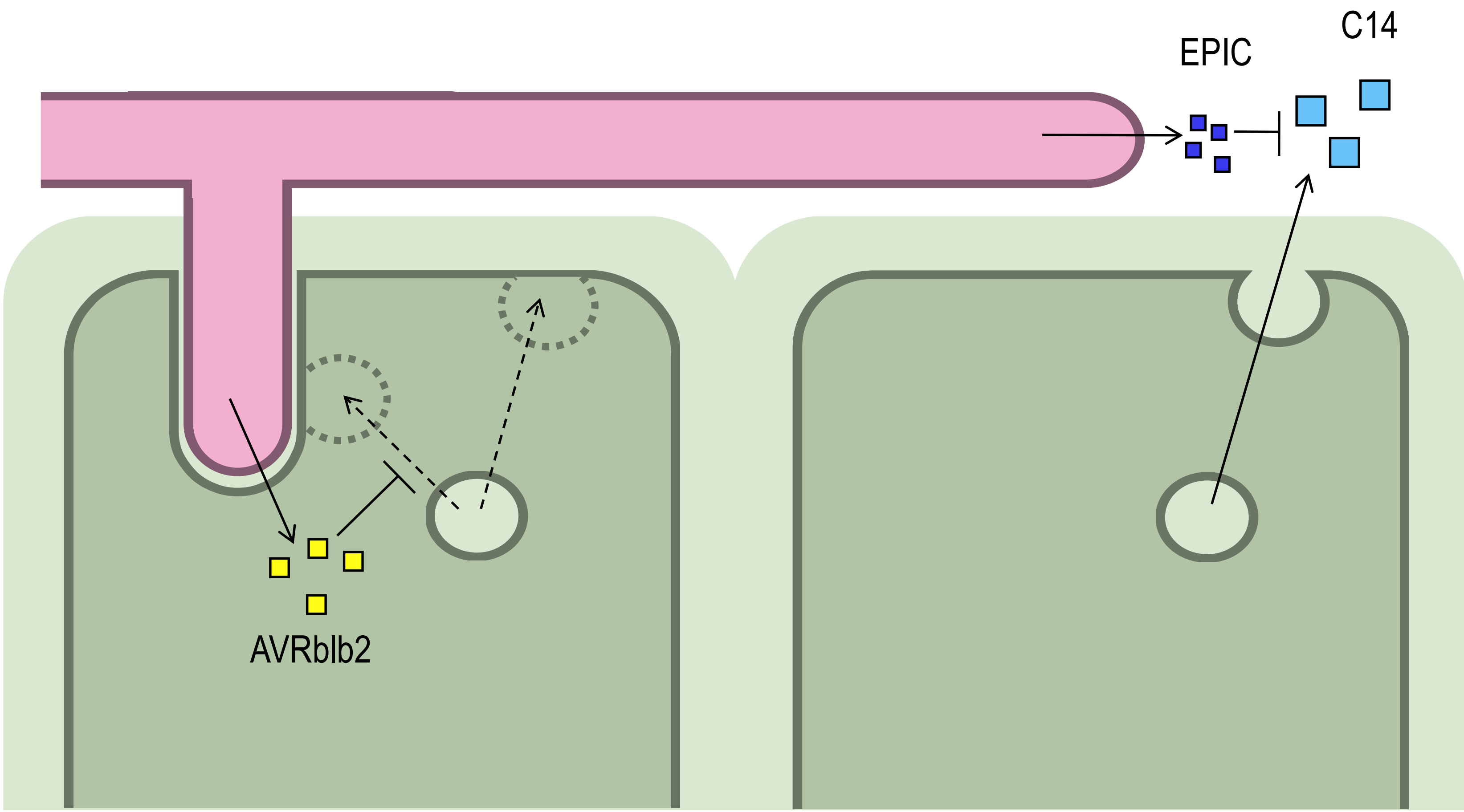


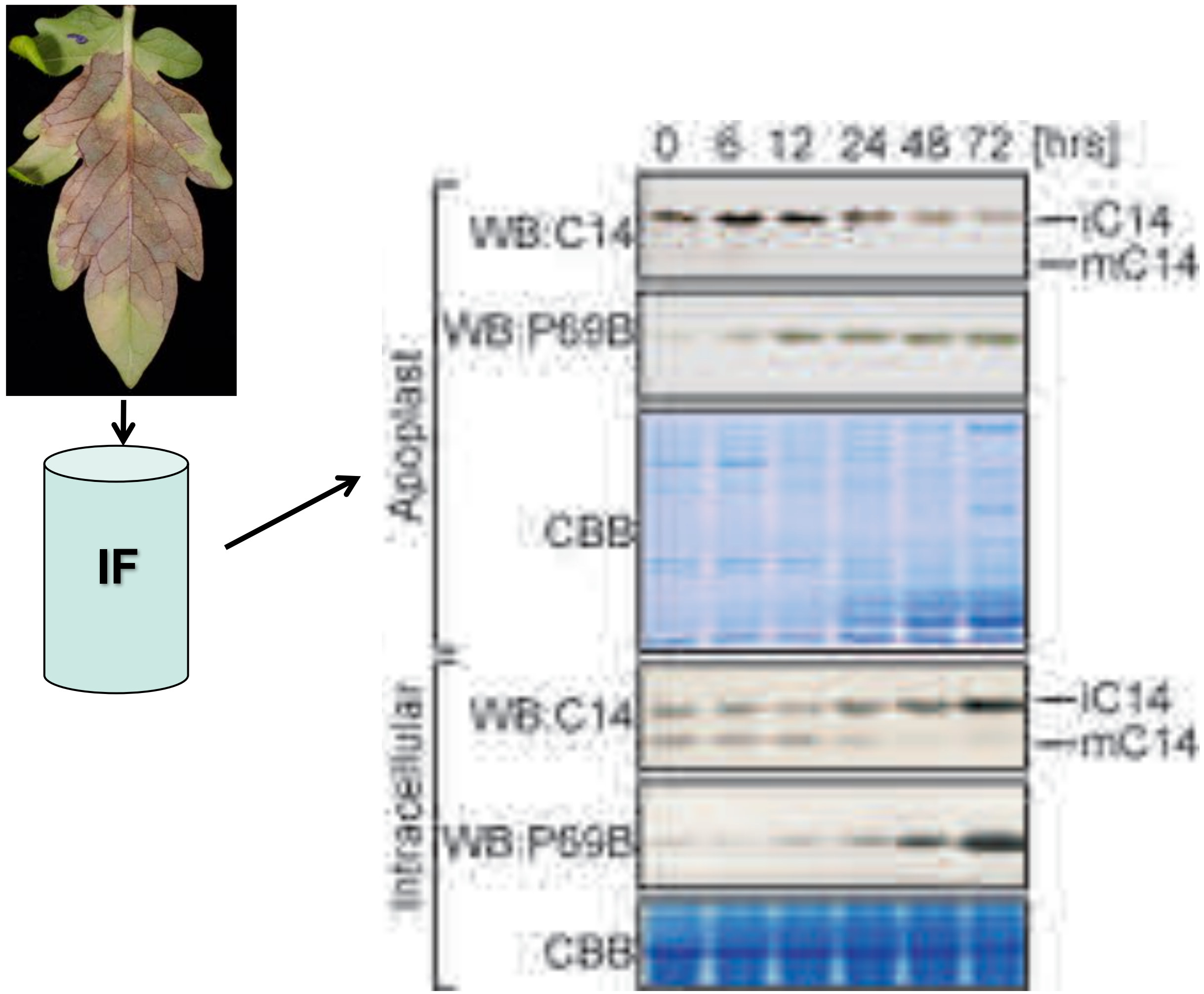
Kamoun & van der Hoorn Labs

P. infestans evolved distinct effectors and mechanisms to interfere with apoplastic proteases



Kamoun & van der Hoorn Labs

Unlike PR proteins, C14 protease is depleted from tomato apoplast during *P. infestans* infection



Bozkurt, Schornack et al. PNAS 2012

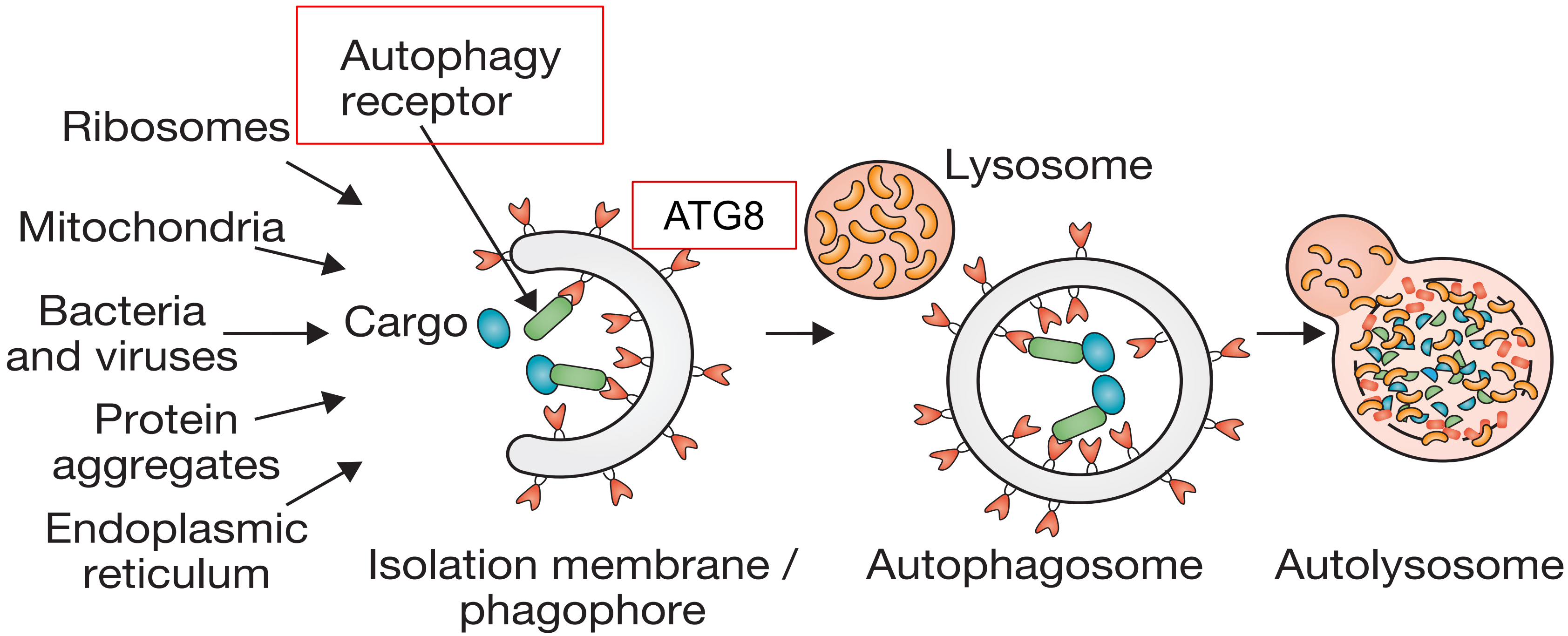
Sophien Kamoun
@KamounLab

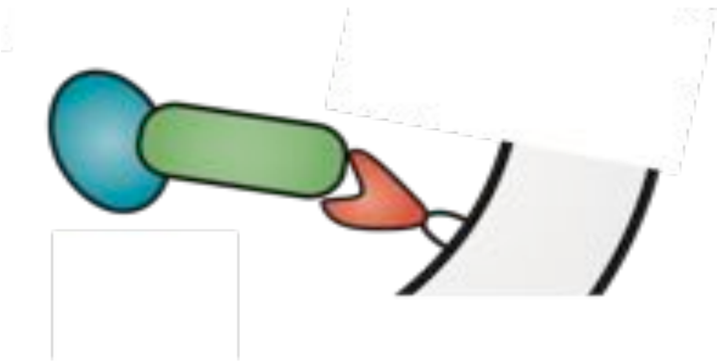
Working on an ATG8-PexRD54 talk while in wagon 8, seat 54... This Is #InnerPeace

RETWEET 1 FAVORITES 8

11:50 AM - 27 Apr 2015

Selective autophagy safeguards eukaryotic cells against various stress conditions

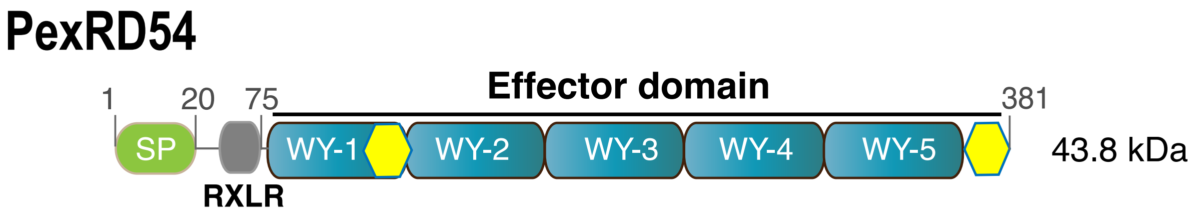




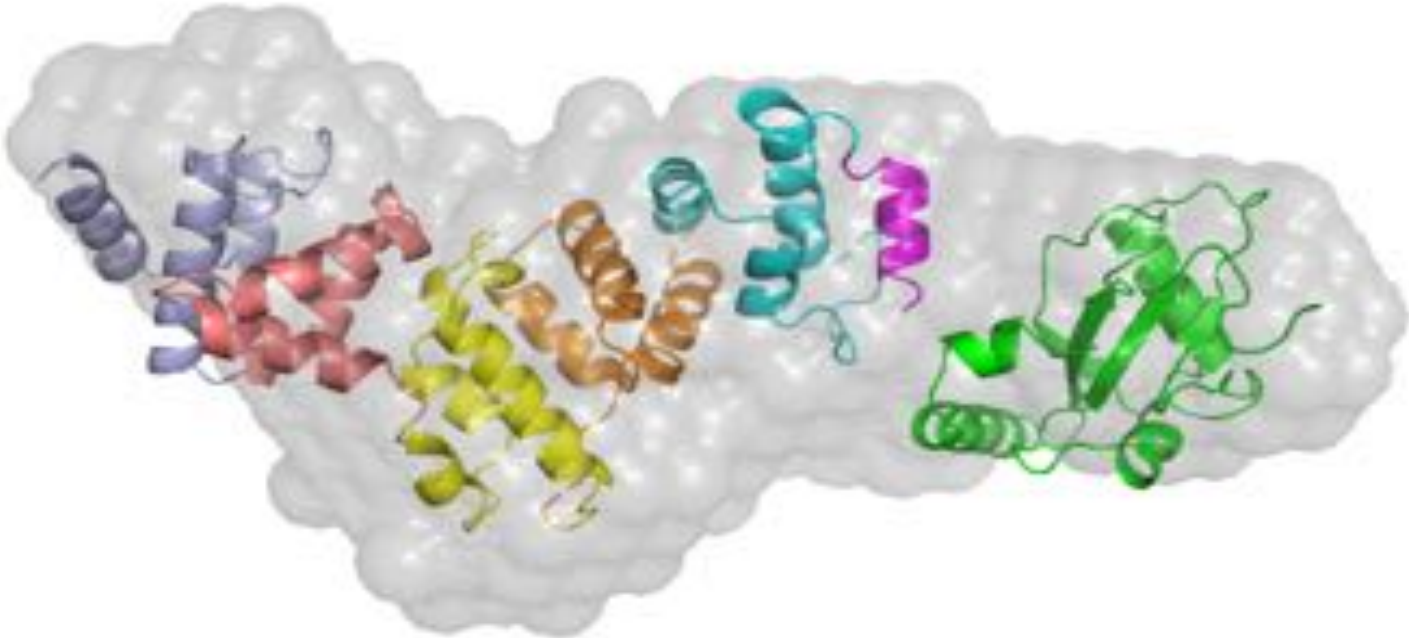
Cargo receptors bind ATG8 via ATG8 Interacting Motif (AIM) = (W/F/Y)XX(L/I/V)

Stolz et al. 2014 Nat Cell Biol

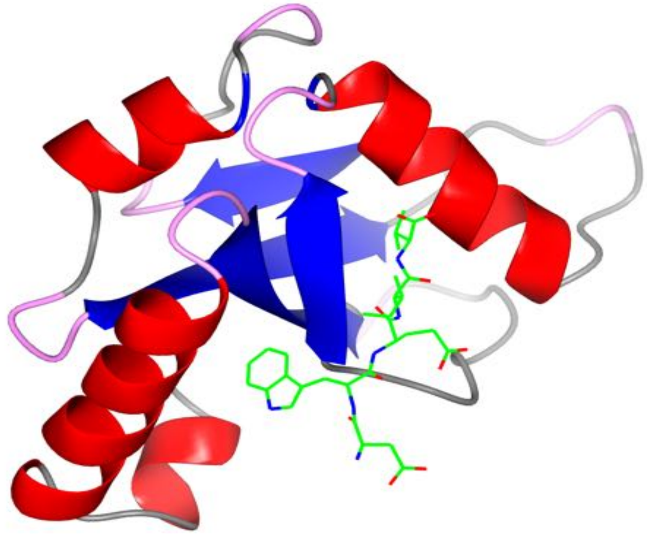
PexRD54 binds ATG8CL via a C-terminal AIM



SAXS Envelope of the complex



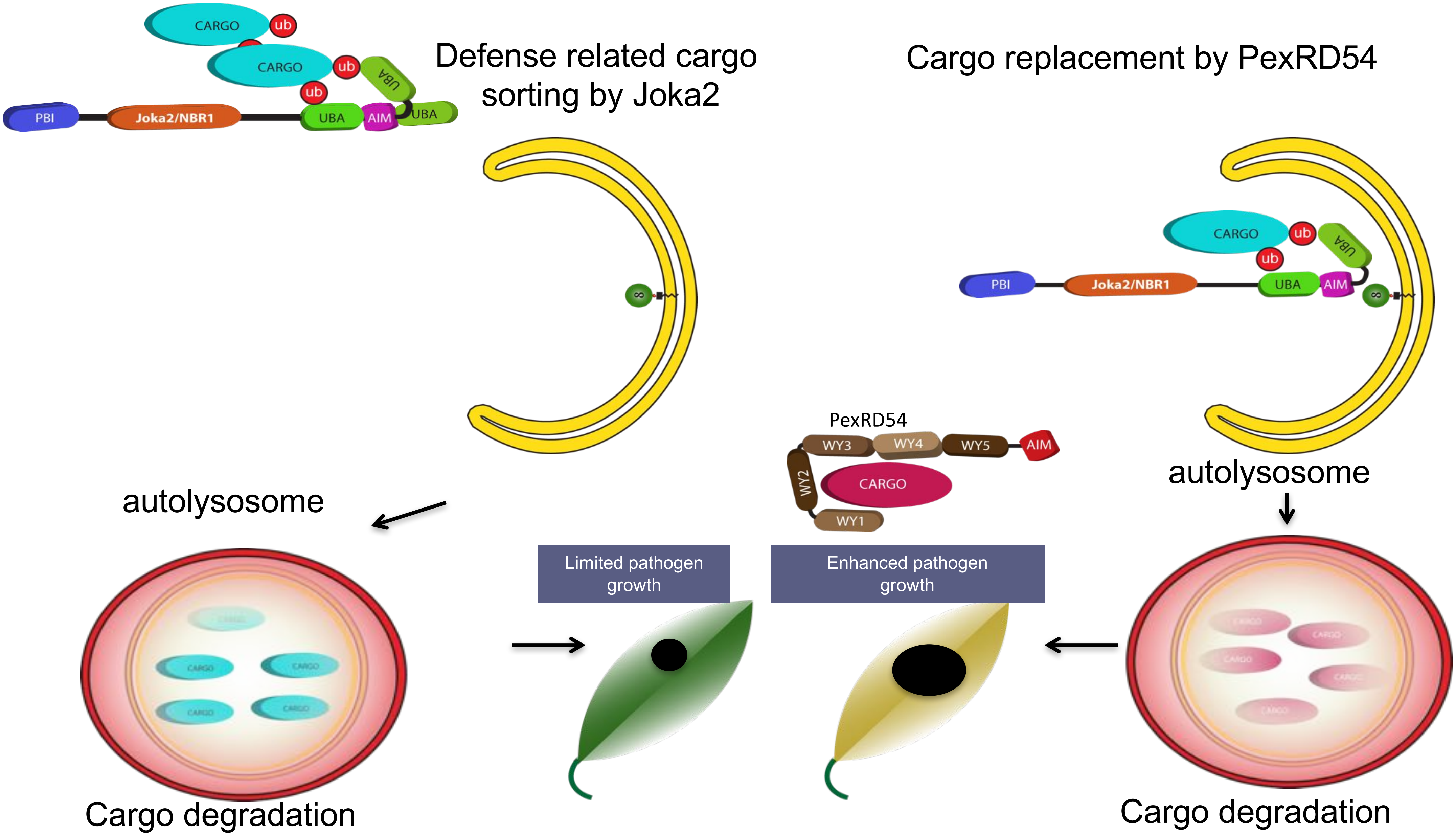
ATG8CL-AIM peptide complex



Abbas Maqbool et al. JBC 2016

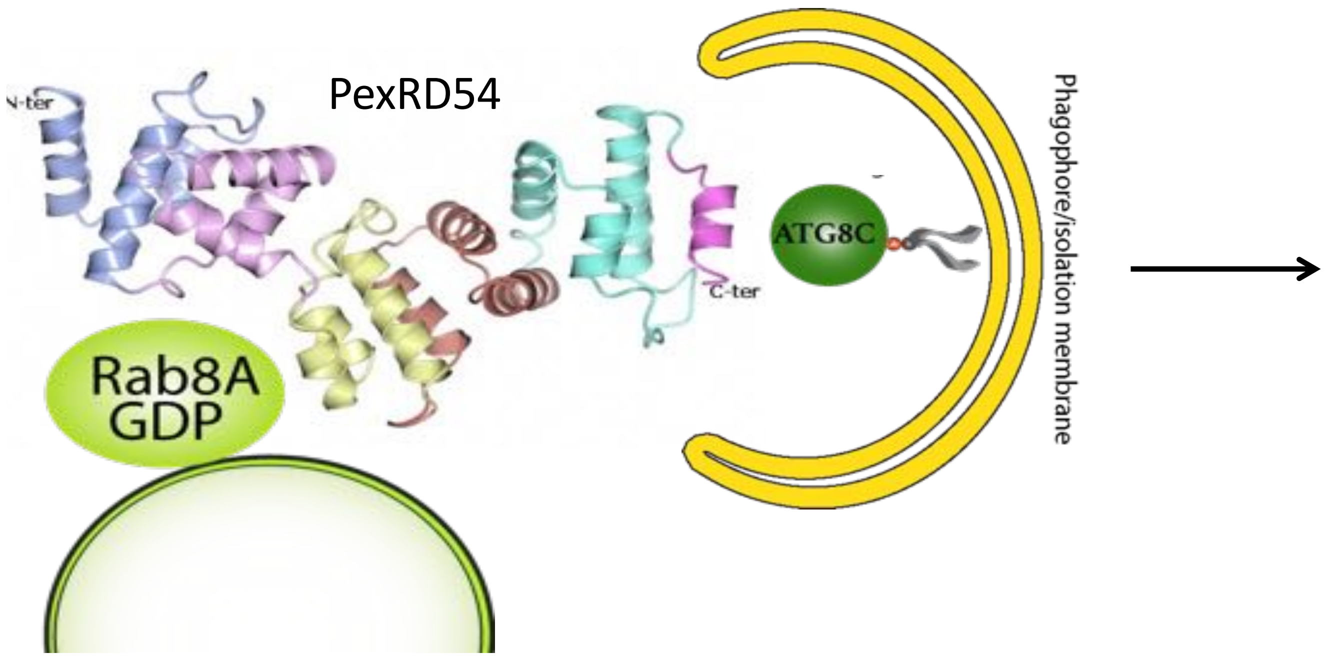
Mark Banfield Lab

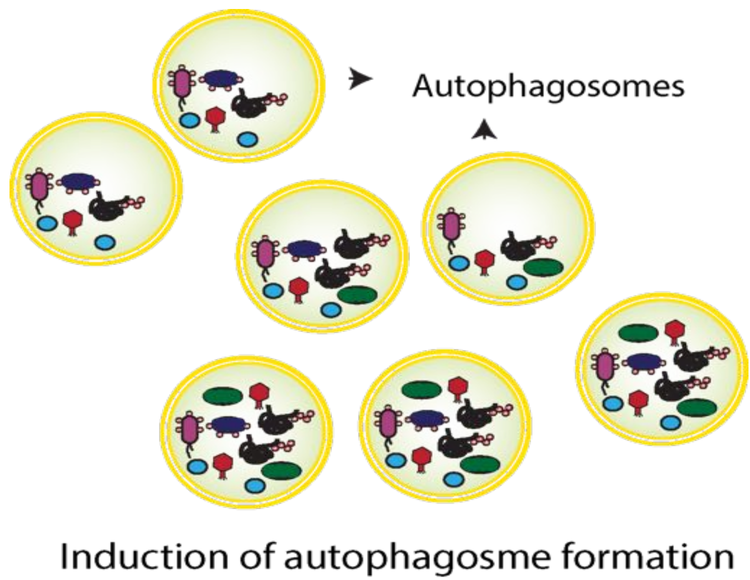
PexRD54 antagonizes the host cargo receptor Joka2 to counteract host defenses



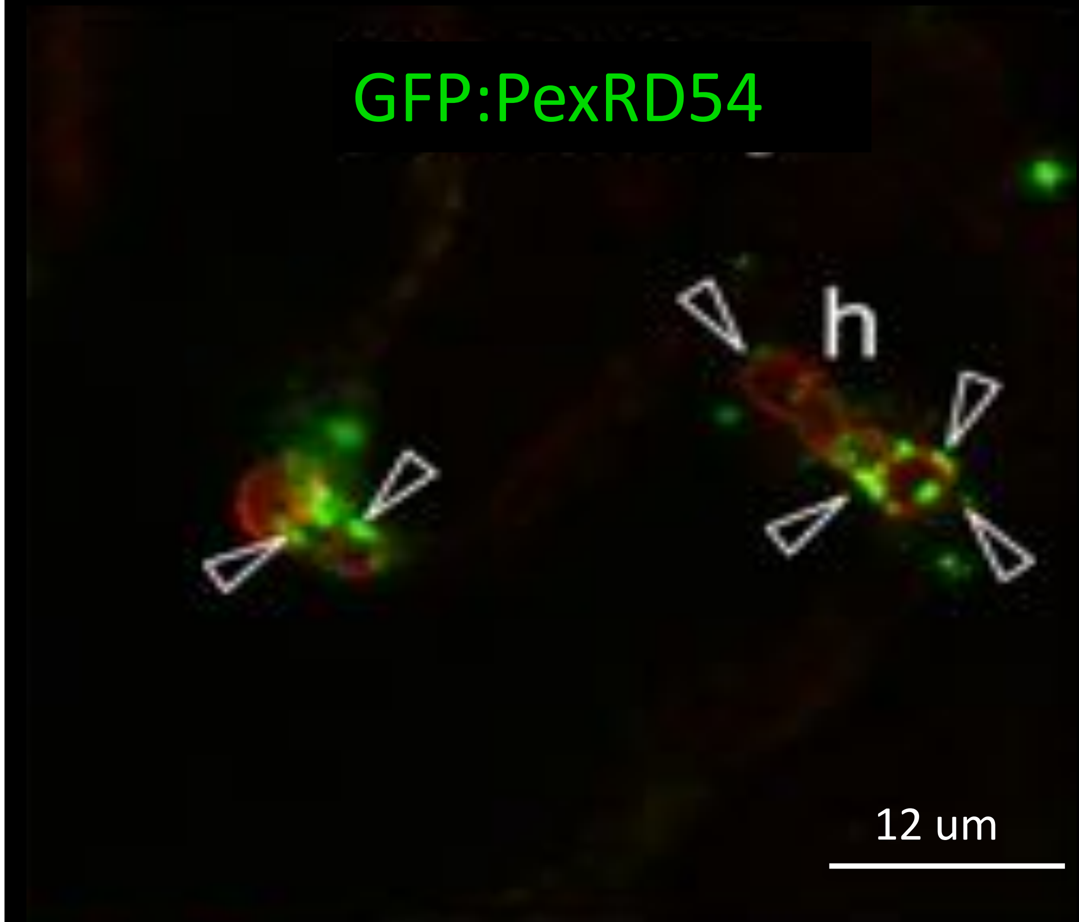
Working model: PexRD54 many effects on autophagy

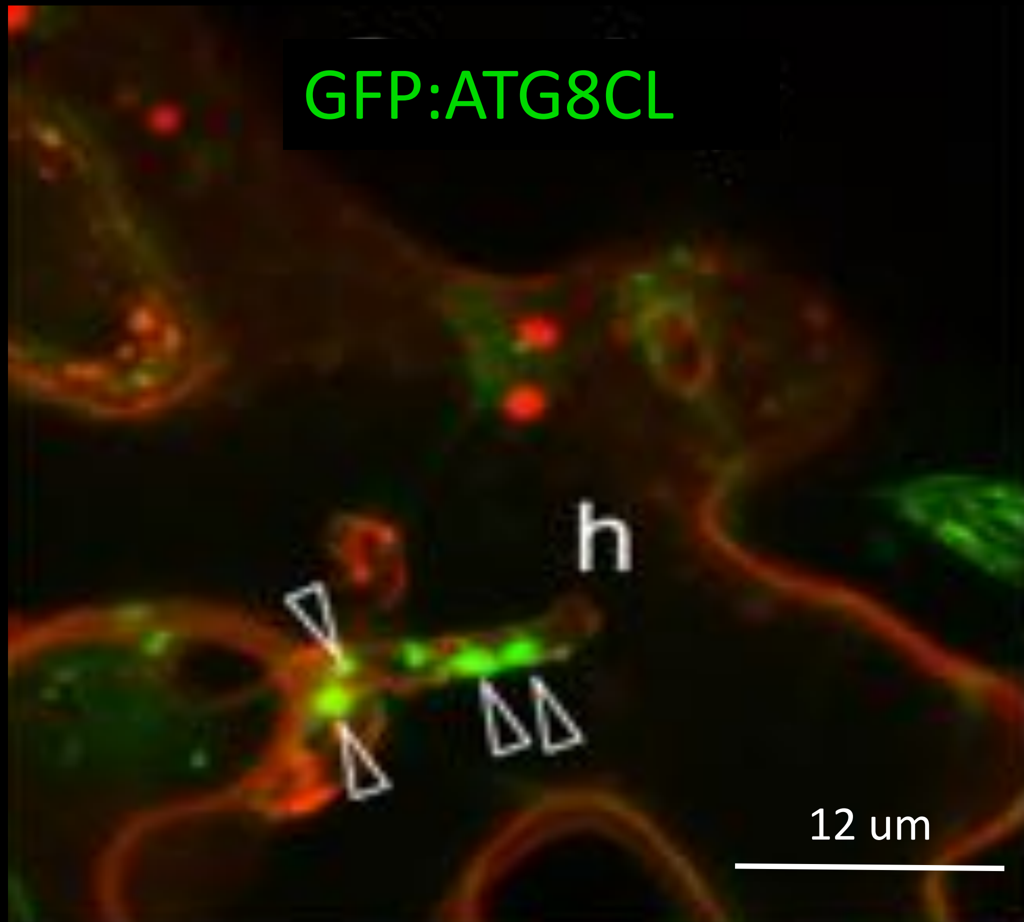
- …antagonizes host cargo receptor Joka2
- …stimulates and co-opts ATG8CL selective autophagy
- …mimics a cargo receptor
- …rerouts ATG8CL selective autophagy to haustoria



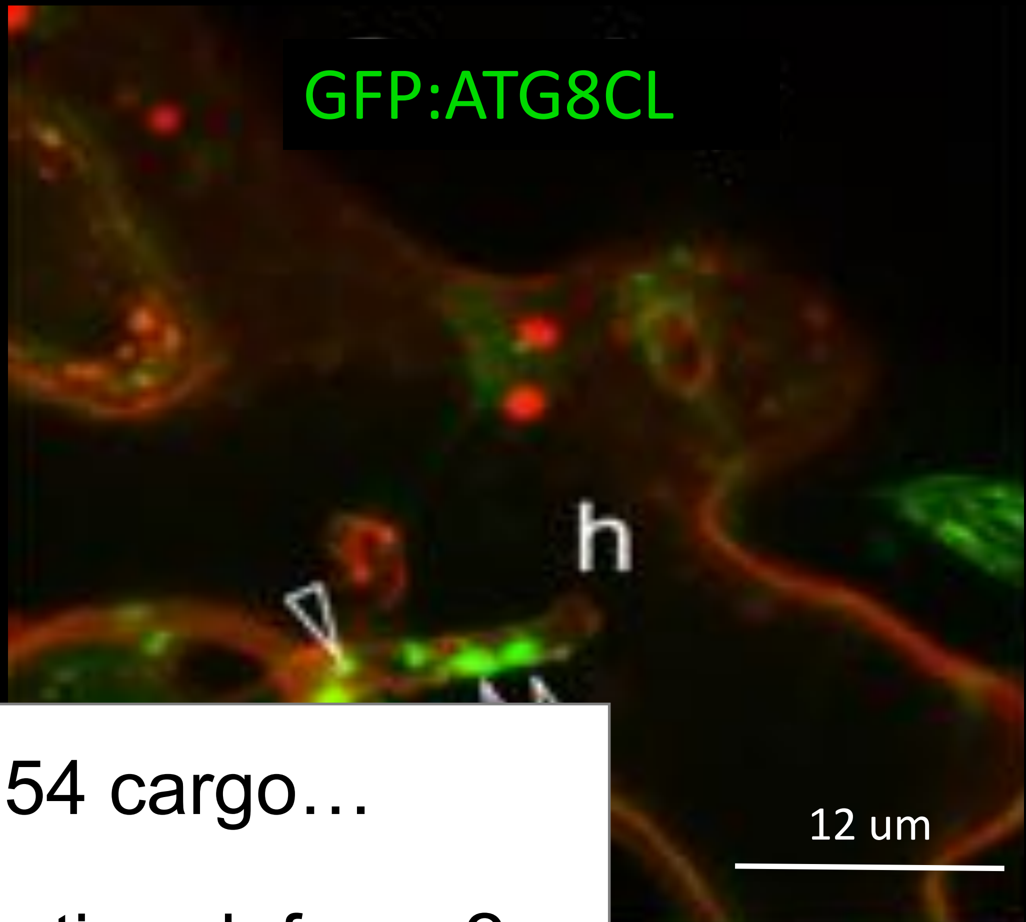


Does PexRD54 hack ATG8CL selective autophagy and divert it to the haustorial interface?





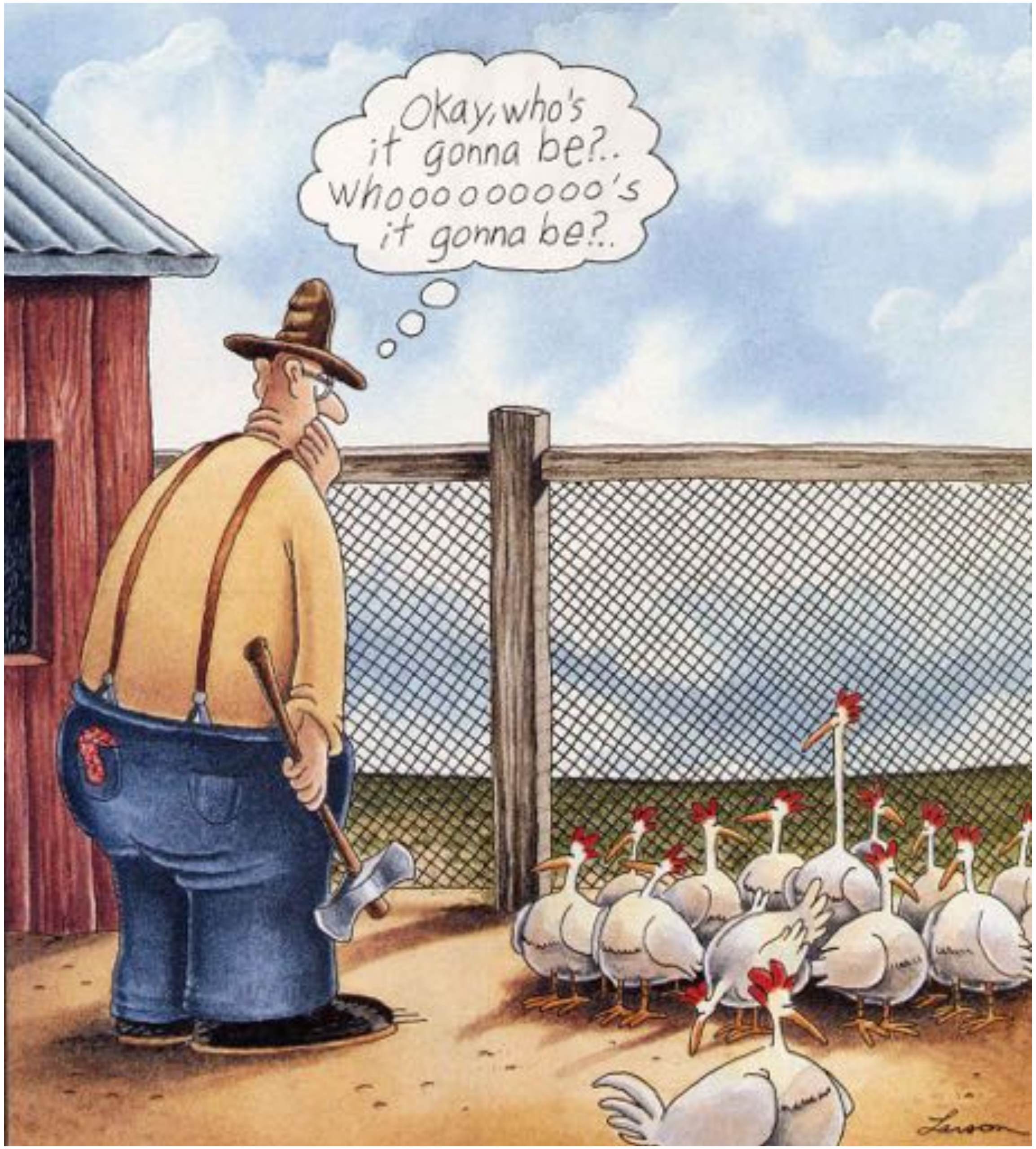
Does PexRD54 hack ATG8CL selective autophagy and divert it to the haustorial interface?



What is PexRD54 cargo…

Nutrition? Counteracting defense?

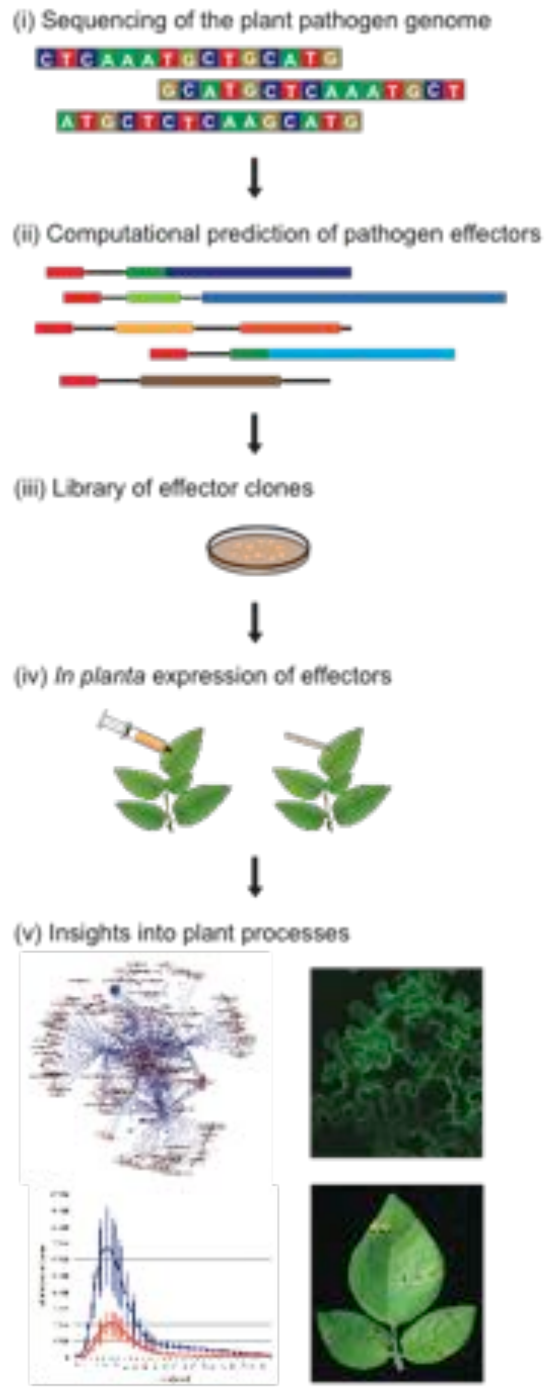
How to identify effector genes?



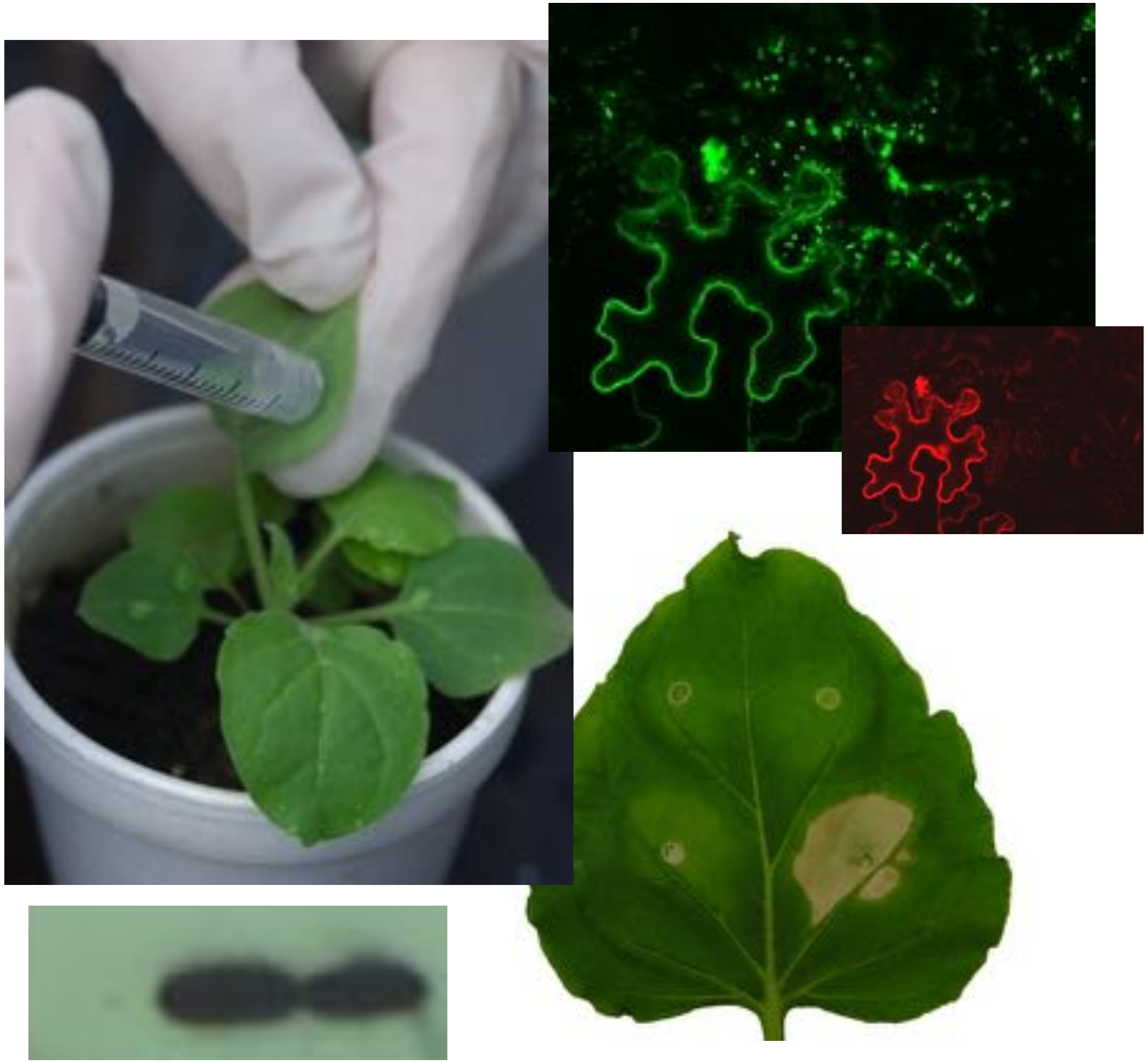
From pathogen genomes to host processes

- "Effectoromics" pipelines to link effectors to plant processes
  - effectors as molecular probes
  - modulation of plant immunity
  - perception by plant immune receptors
  - unexpected findings…

Pais et al. Genome Biology 2013



Nicotiana benthamiana: The ‘HeLa cells’ system of plant biology



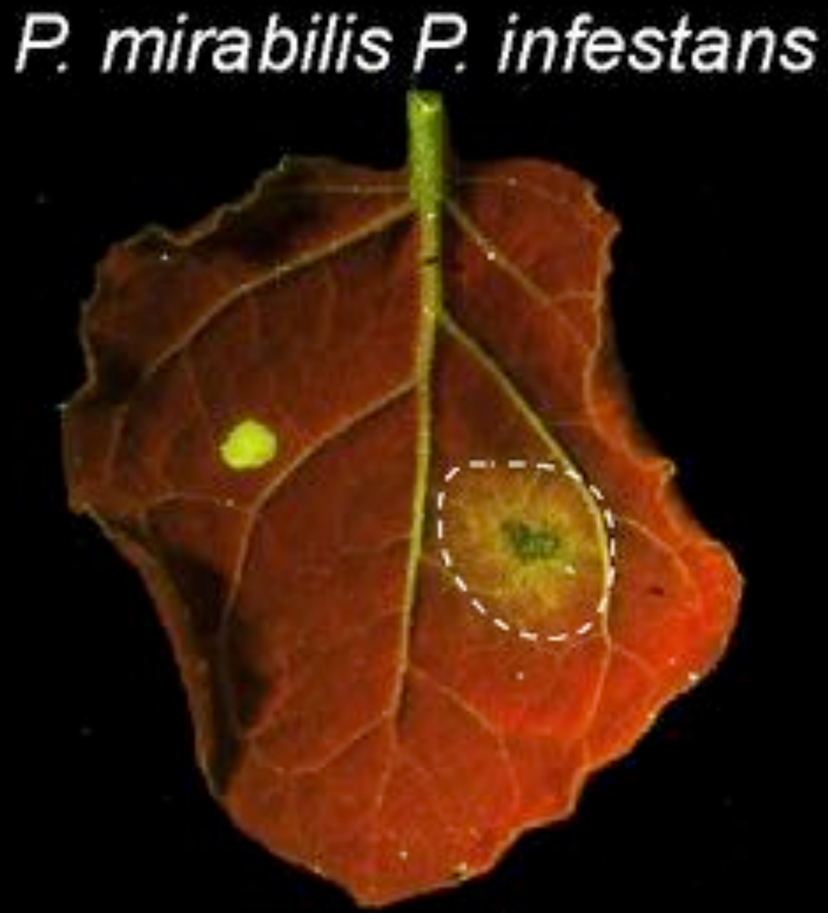
Agroinfiltration

- Virus vectors
- Gene co-expression
- Gene silencing
- Cell biology
- Protein biochemistry
- Protein complexes
- High-throughput screens

Phytophthora infestans infects *Nicotiana benthamiana*

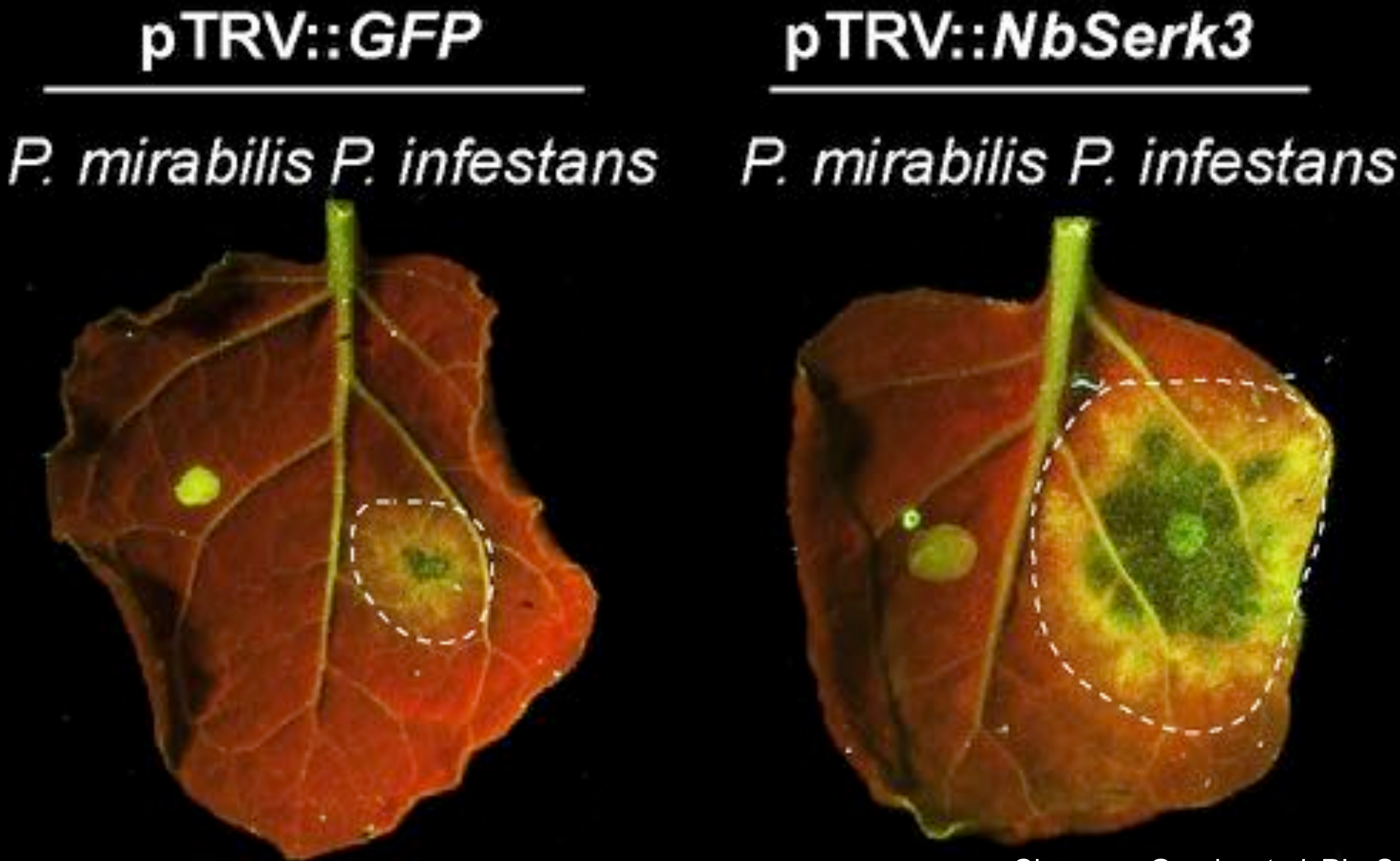
An experimental host system for the late blight disease

agroinfiltration, gene silencing,
protein biochemistry, fast-forward cell biology



Chaparro-Garcia *et al*. PLoS ONE 2011

SERK3/BAK1 is required for basal immunity against *Phytophthora infestans* in *Nicotiana benthamiana*

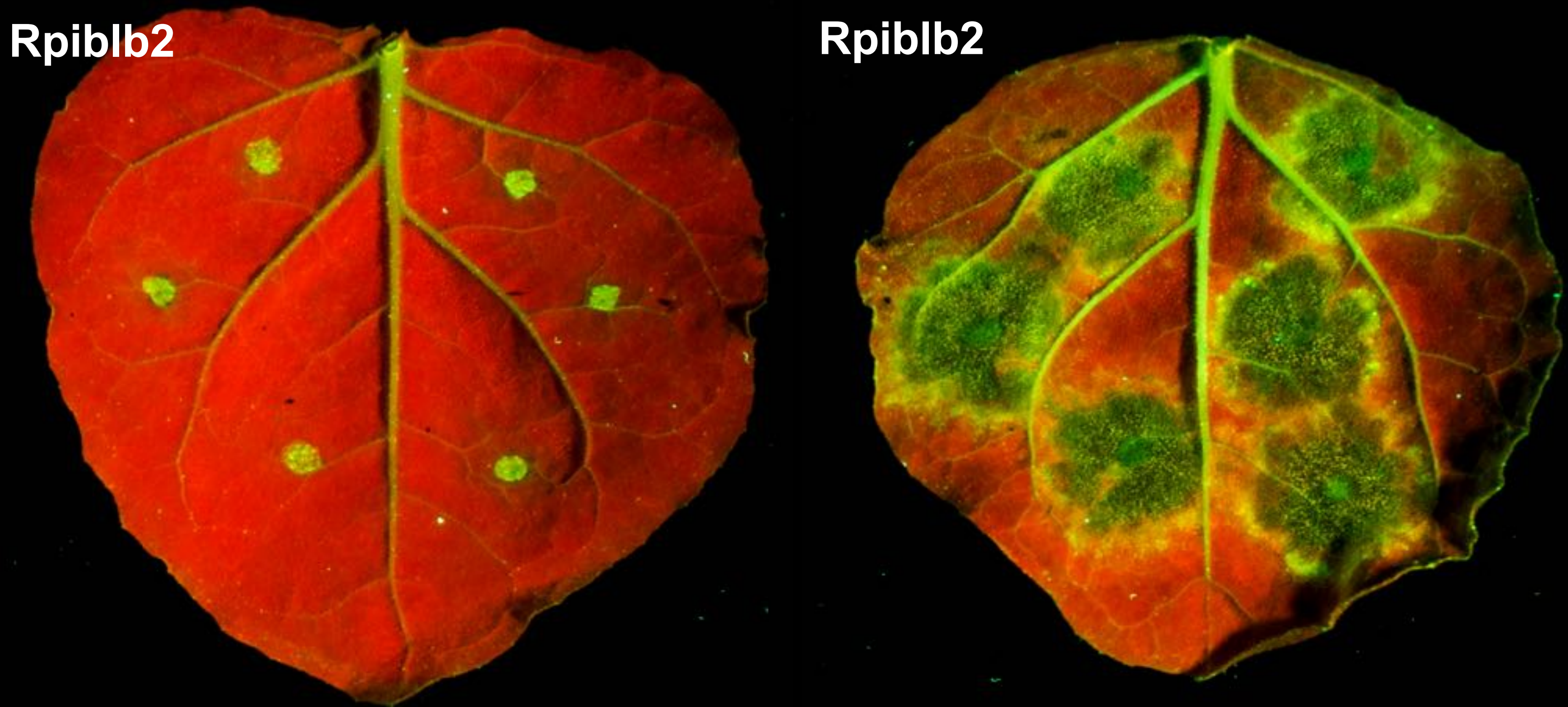


Chaparro-Garcia *et al*. PLoS ONE 2011

Potato NLRs function in benth!

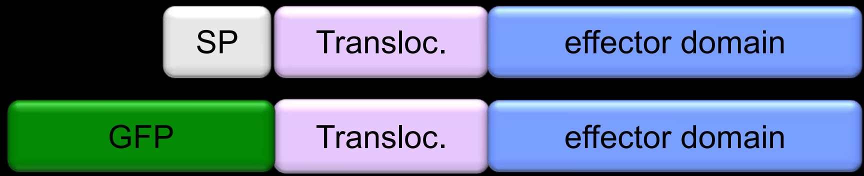
NRC4 is required for Rpiblb2-mediated resistance
(virus-induced gene silencing with *Tobacco rattle virus* – TRV)

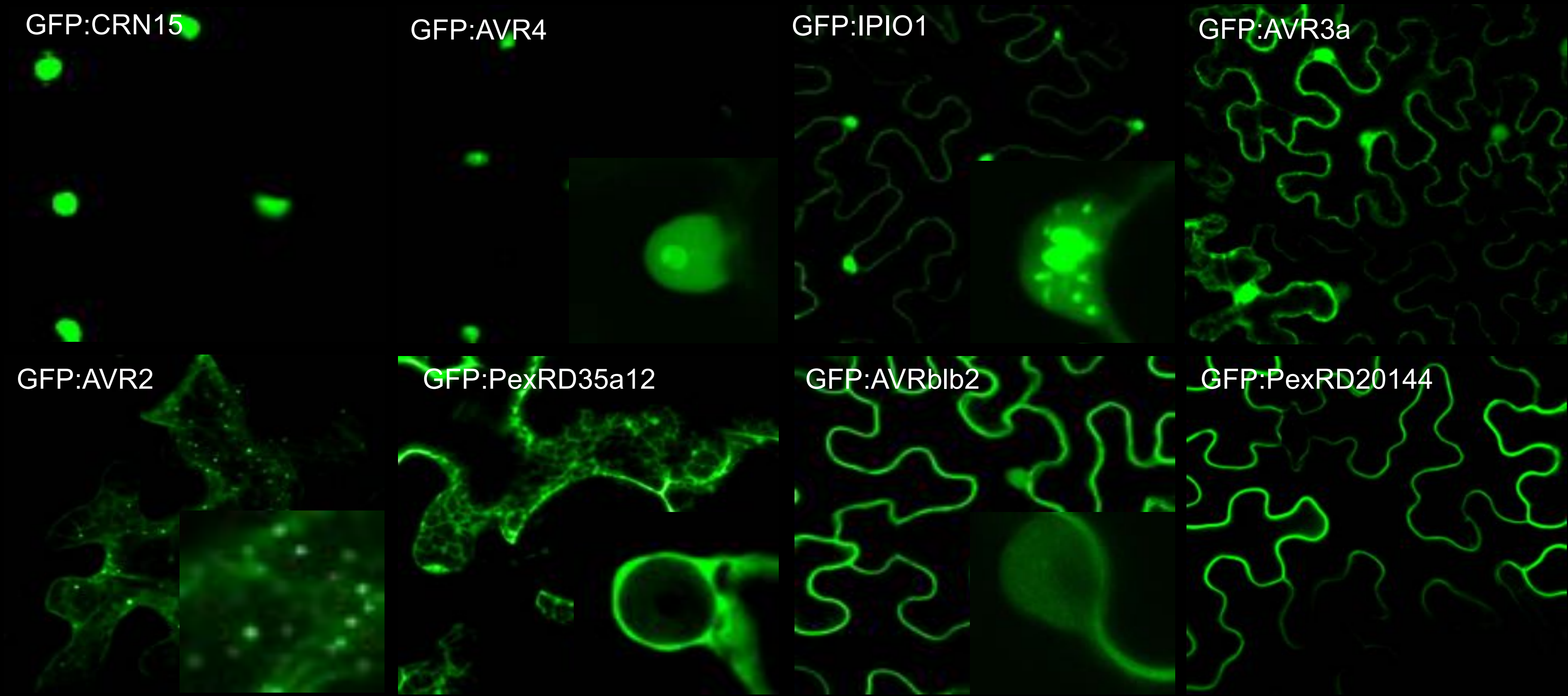
pTRV:GFP **pTRV:NRC4**



Wu et al. PNAS 2017

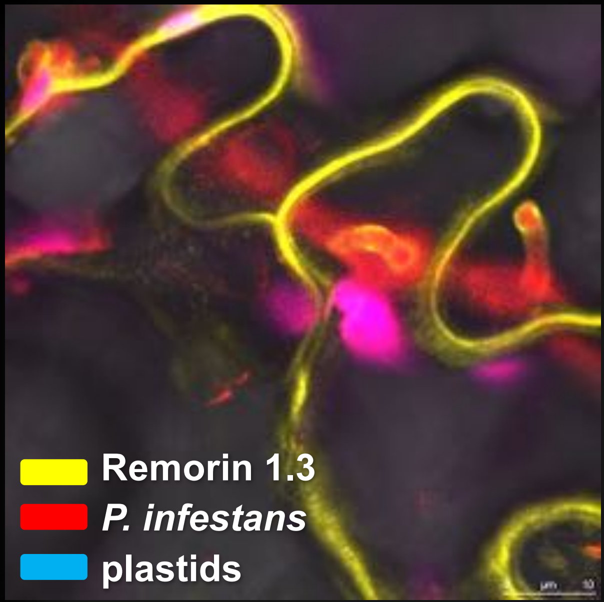
Quo vadis, effector?
Fast-forward plant cell biology

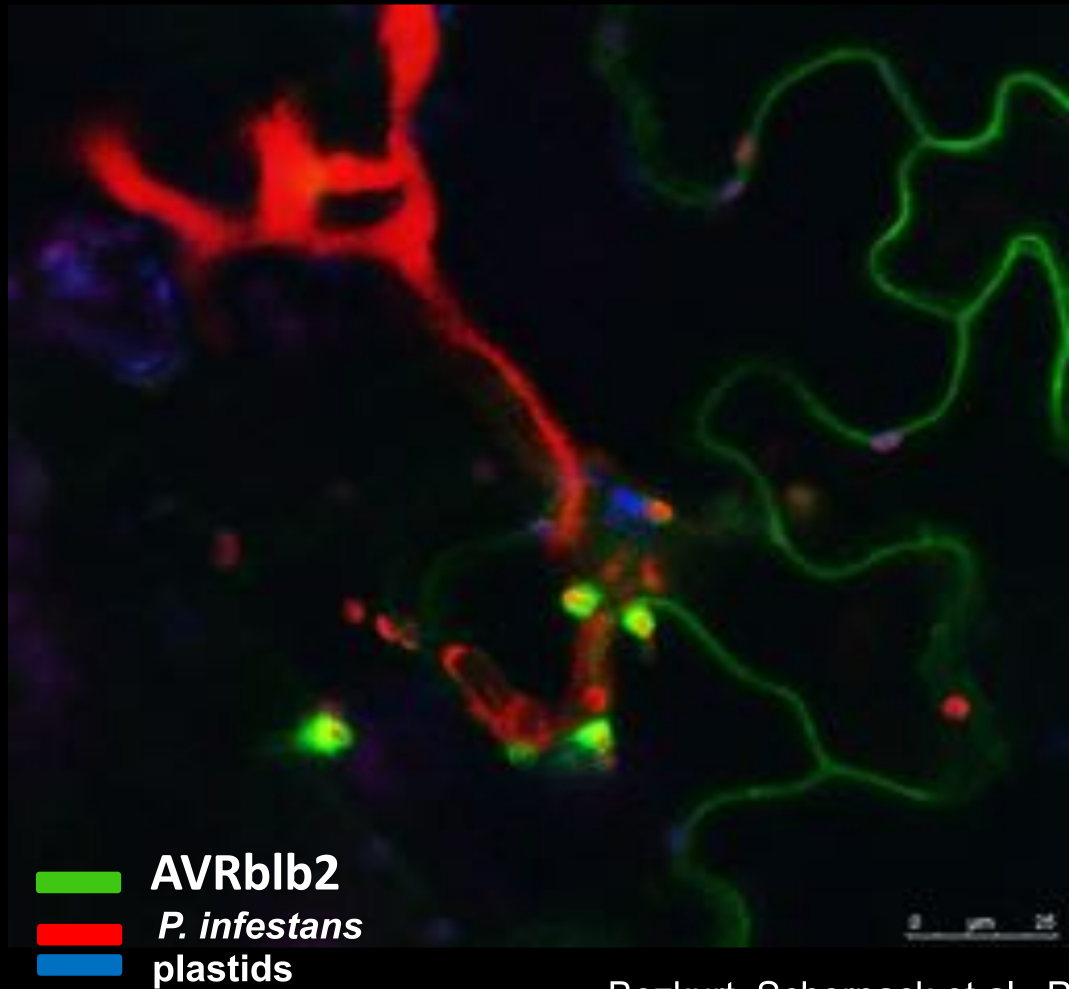




Schornack et al (2010), PNAS; Bozkurt, Schornack et al. (2011) PNAS and unpublished

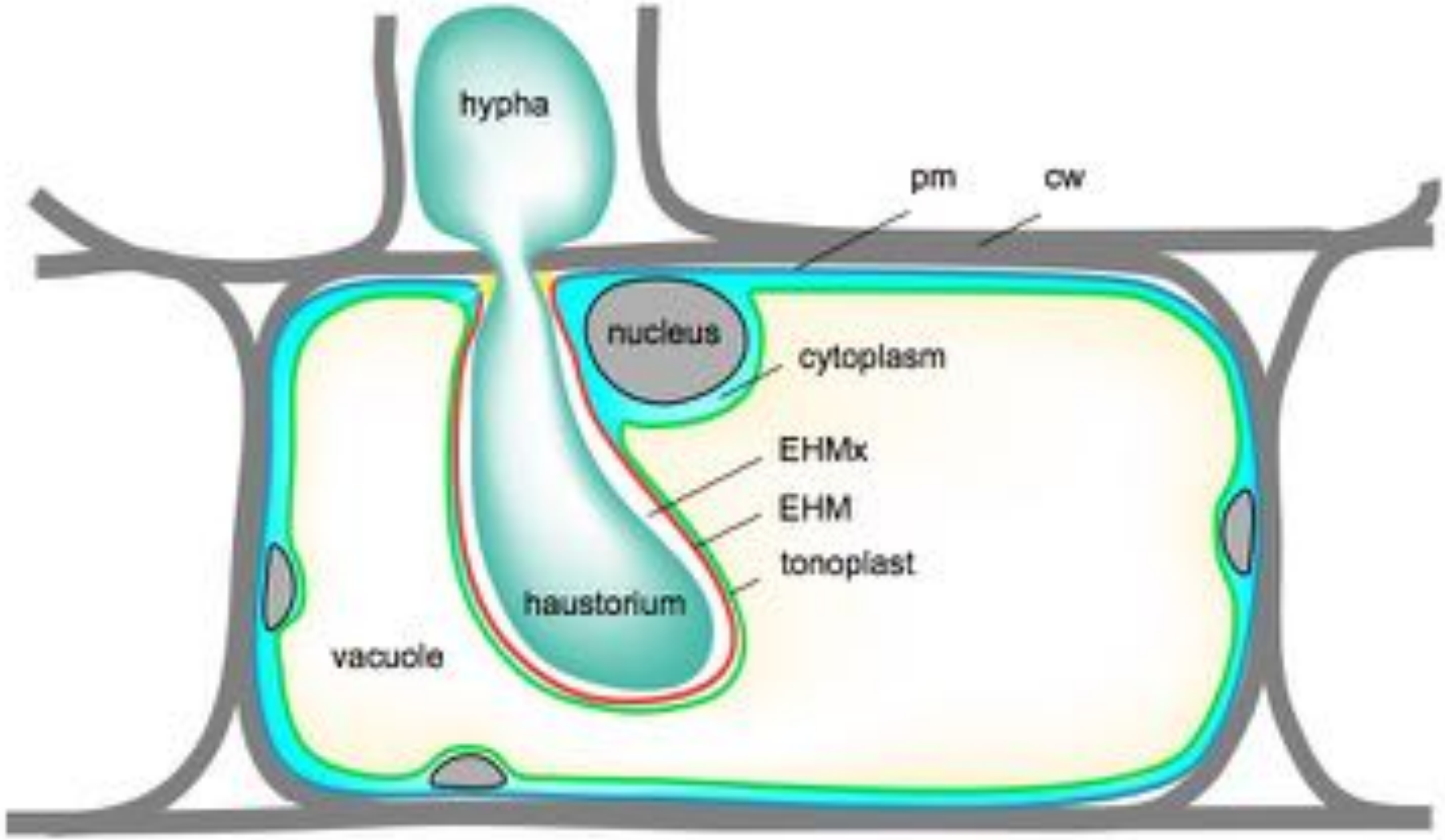
Fast-forward cell biology of haustoriated plant cells





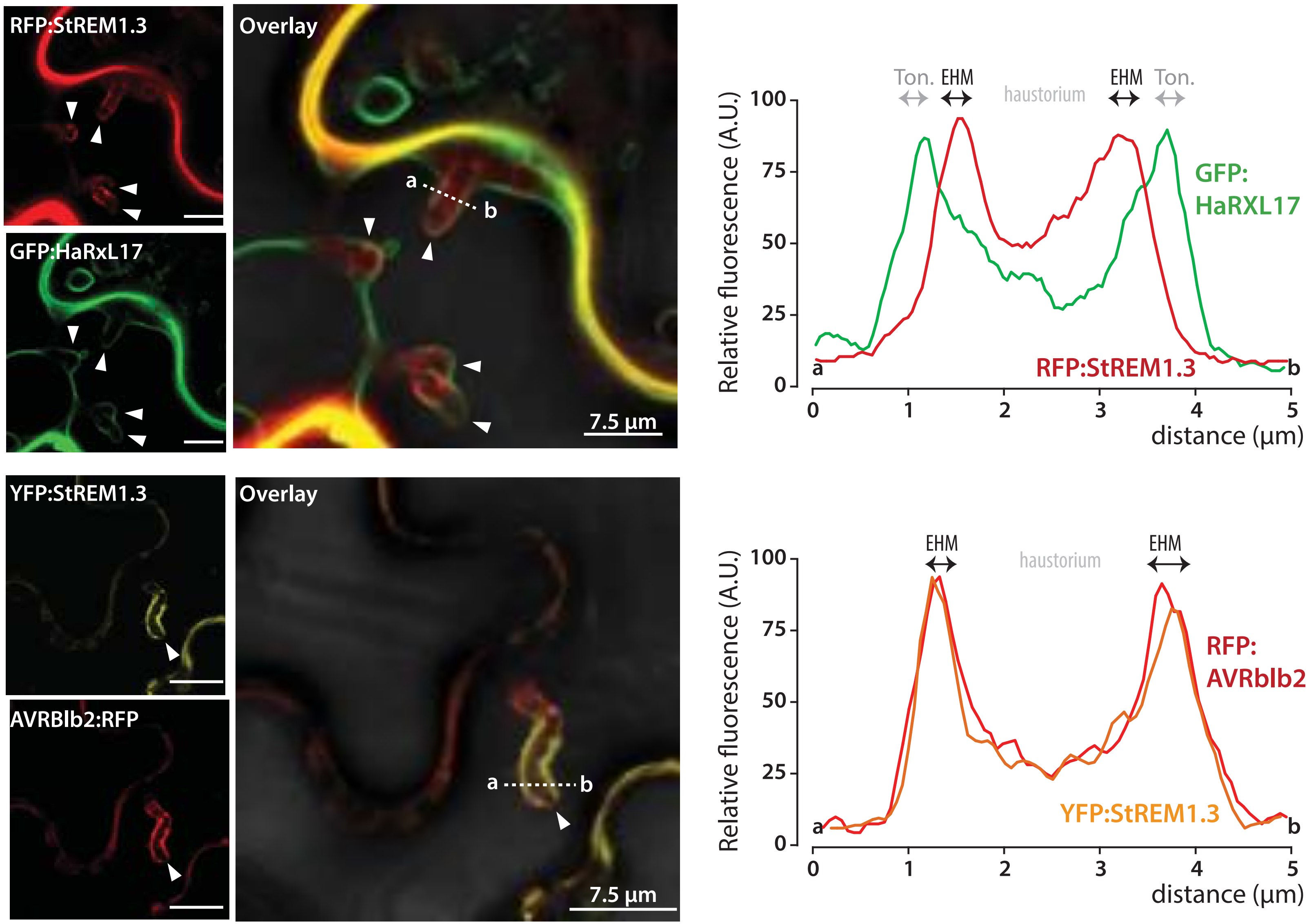
Bozkurt, Schornack et al., PNAS 2011
Lu et al., Cell Microbiol 2012
Saunders et al., Plant Cell 2012
Bozkurt et al., Plant Phys 2014
Bozkurt et al., Traffic 2015
Dagdas, Belhaj et al., eLife 2016

Layers of compartments surround the haustorium in the infected plant cell



- Can we use effectors as probes for haustorial cell biology?

RXLR effectors AVRblb2 and HaRXL17 as subcellular markers

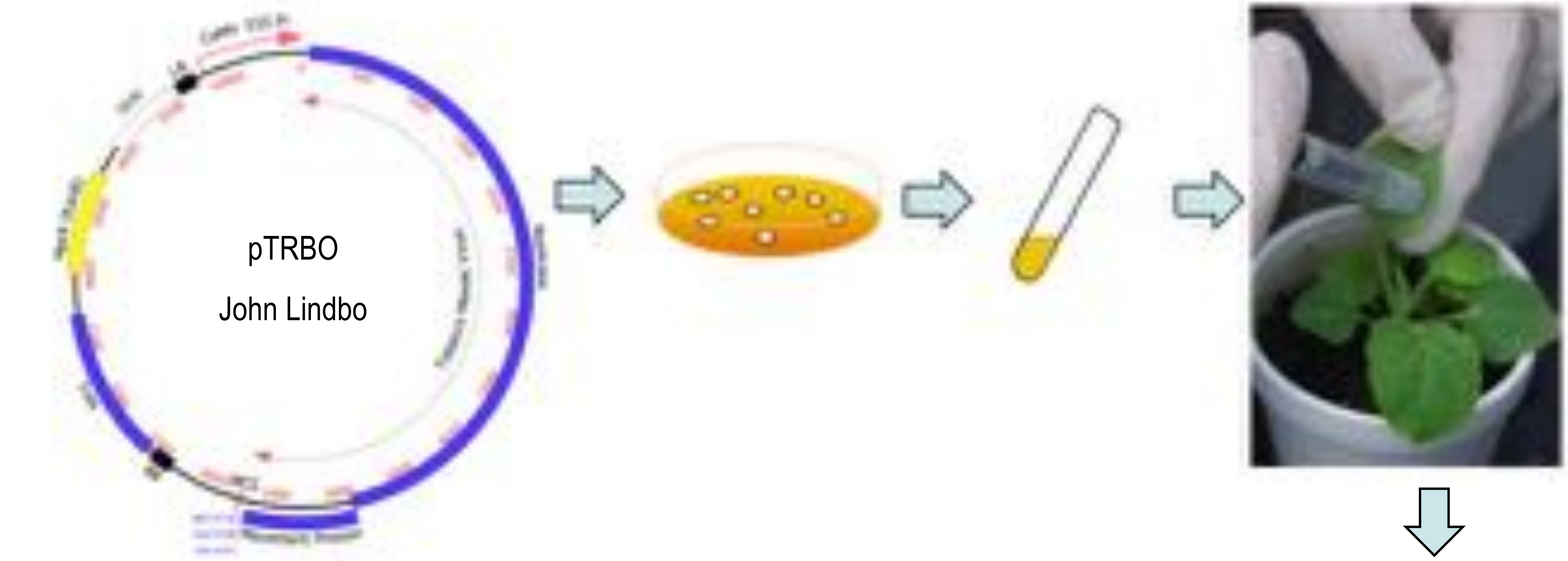


AVRblb2 and StREM1.3 in EHM

HaRXL17 in tonoplast

Tolga Bozkurt, Sylvain Raffaele Plant Phys 2014 – thanks to MC Caillaud (J Jones Lab)

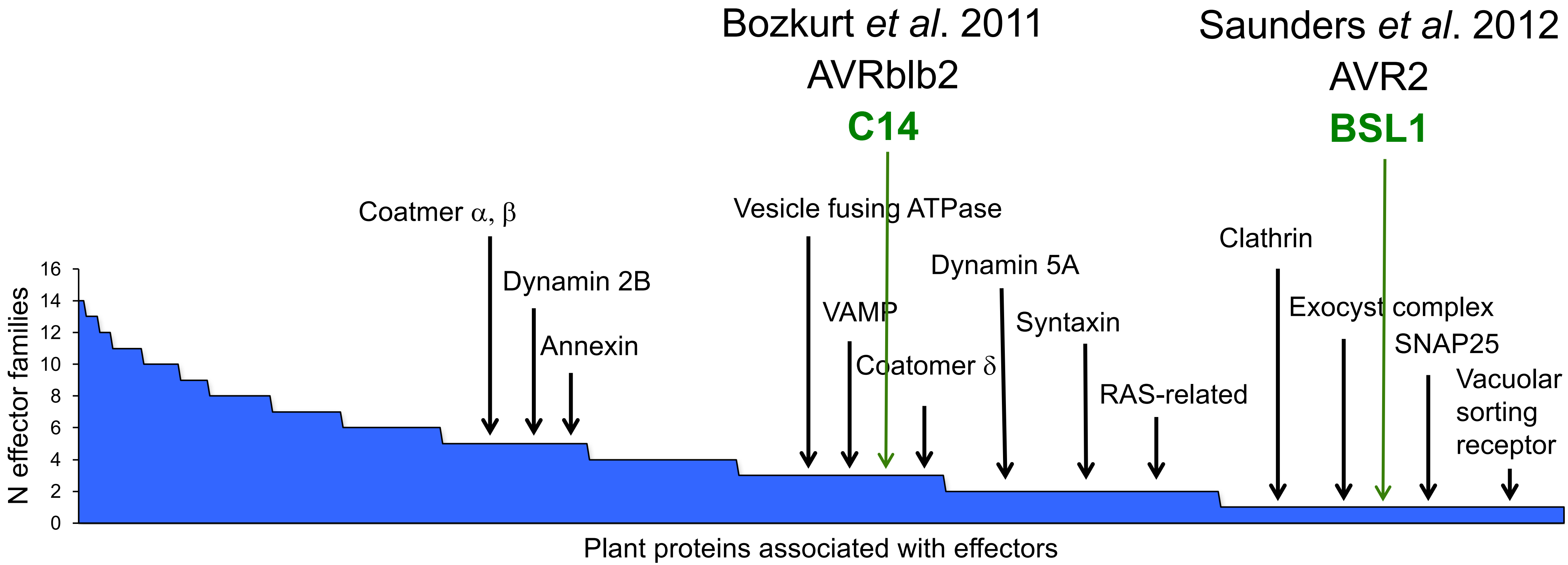
in planta effector-target protein complexes



- co-IPs using anti-FLAG resins
- Mass-Spectrometry (LC-MS/MS)
- Match to customized database
- >70 RXLR effectors tested

Win, J., Kamoun, S., Jones, A.M. Method Mol Biol 2011

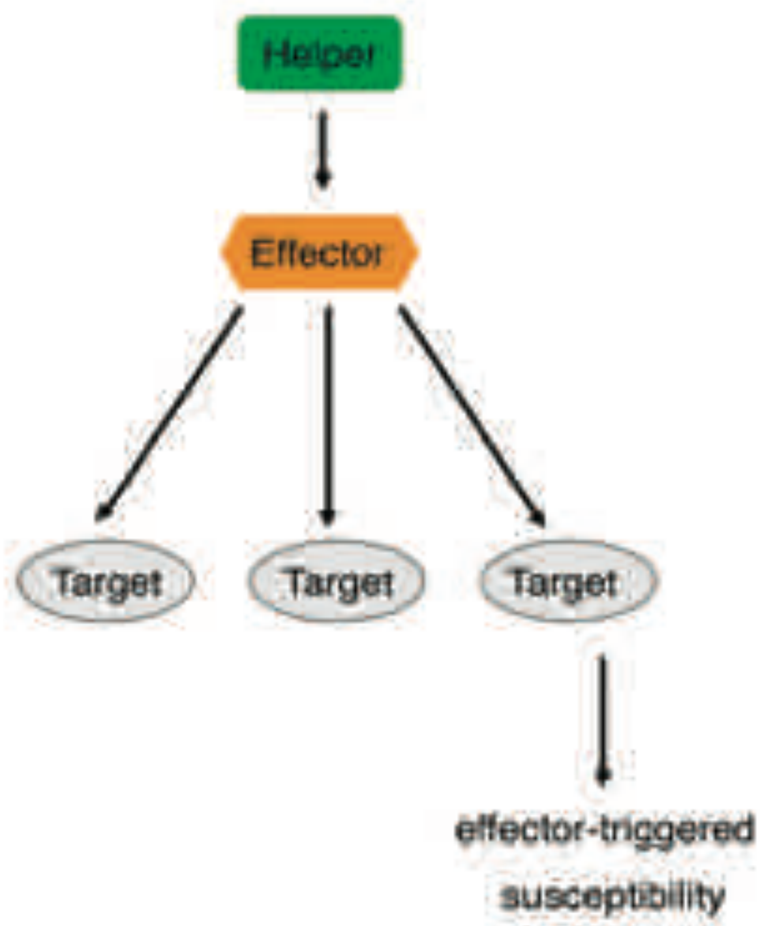
Co-IP screen reveals plant protein complexes associated with *P. infestans* RXLR effectors

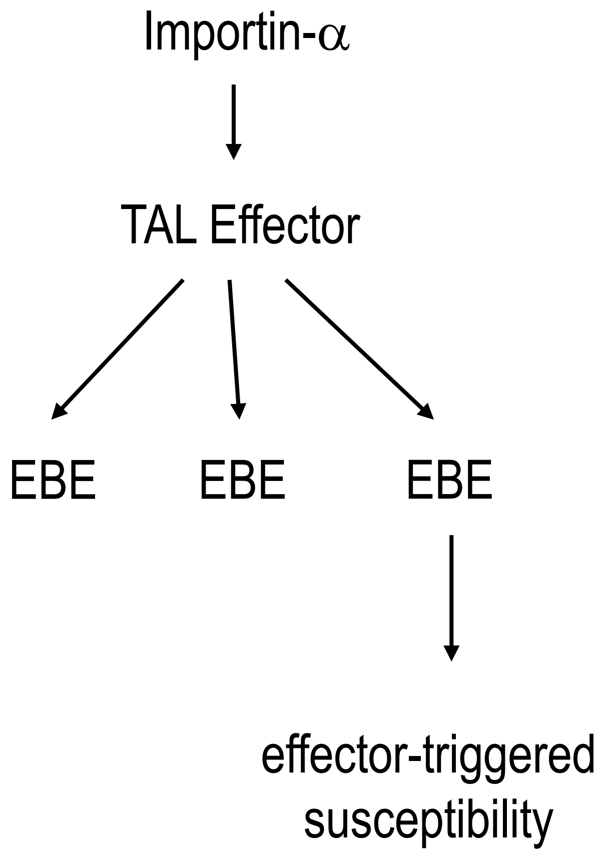


- Many interactors involved in vesicle trafficking
- Could be *effector helpers/facilitators* or *targets* (Win et al. CSHSQB 2013)

Joe Win, Jan Skelnar, Alex Jones

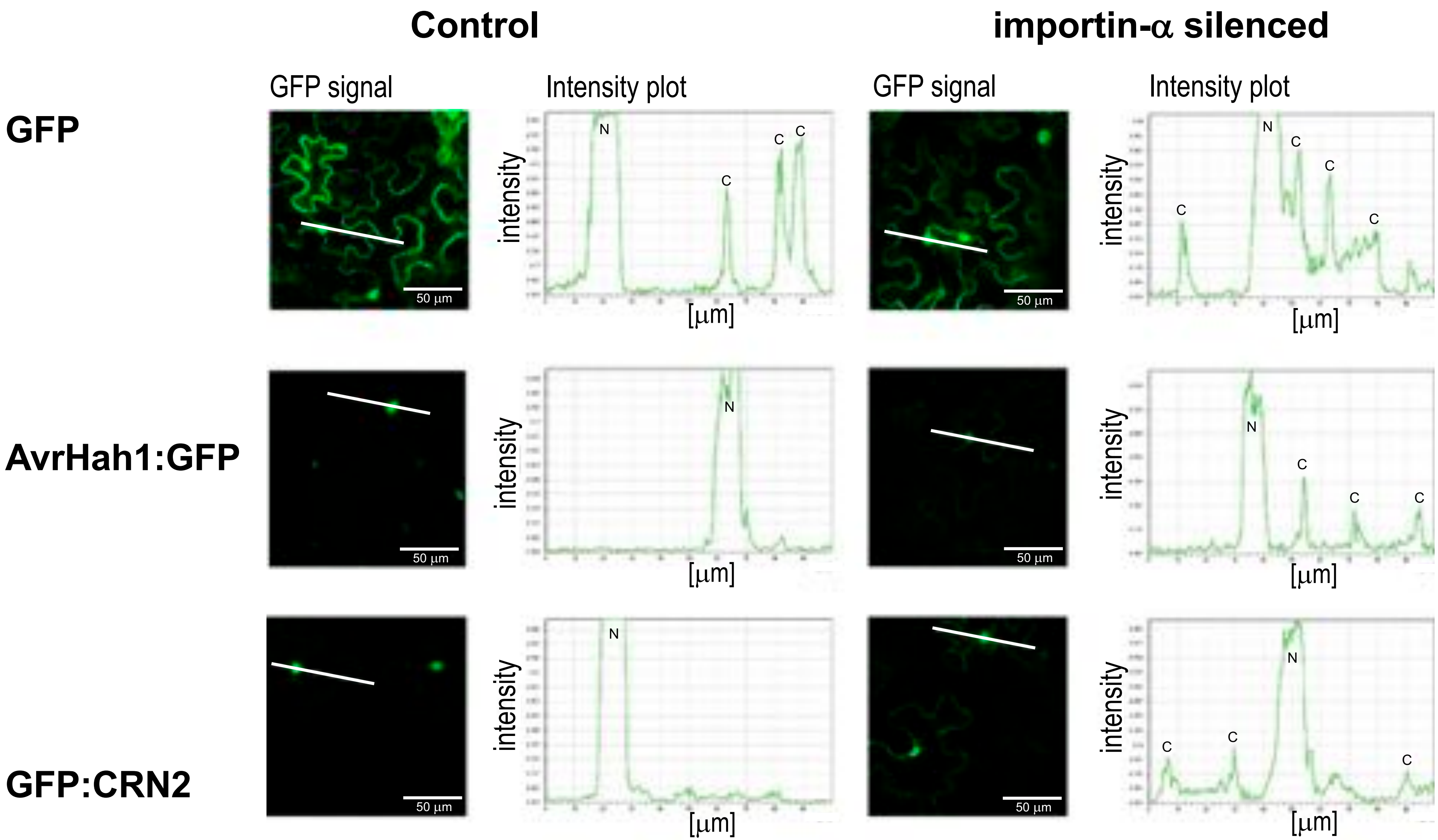
Host S factors: helpers (facilitators) vs. targets





***Xanthomonas* TAL effectors**

Silencing of importin-α impairs nuclear accumulation of effectors



Schornack, van Damme *et al.* PNAS 2011; based on assay of Kanneganti *et al.* Plant J. 2007

MPMI Vol. 22, No. 2, 2009, pp. 115–122. doi:10.1094/MPMI-22-2-0115. © 2009 The American Phytopathological Society

2009

CURRENT REVIEW

Emerging Concepts in Effector Biology of Plant-Associated Organisms

Saskia A. Hogenhout,[1] Renier A. L. Van der Hoorn,[2] Ryohei Terauchi,[3] and Sophien Kamoun[4]

Effector Biology of Plant-Associated Organisms: Concepts and Perspectives

2012

J. Win,[1] A. Chaparro-Garcia,[1] K. Belhaj,[1] D.G.O. Saunders,[1] K. Yoshida,[1] S. Dong,[1] S. Schornack,[1] C. Zipfel,[1] S. Robatzek,[1] S.A. Hogenhout,[2] and S. Kamoun[1]
[1]*The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH United Kingdom*
[2]*Department of Cell and Developmental Biology, The John Innes Centre, Norwich Research Park, Norwich, NR4 7UH United Kingdom*
Correspondence: sophien.kamoun@tsl.ac.uk

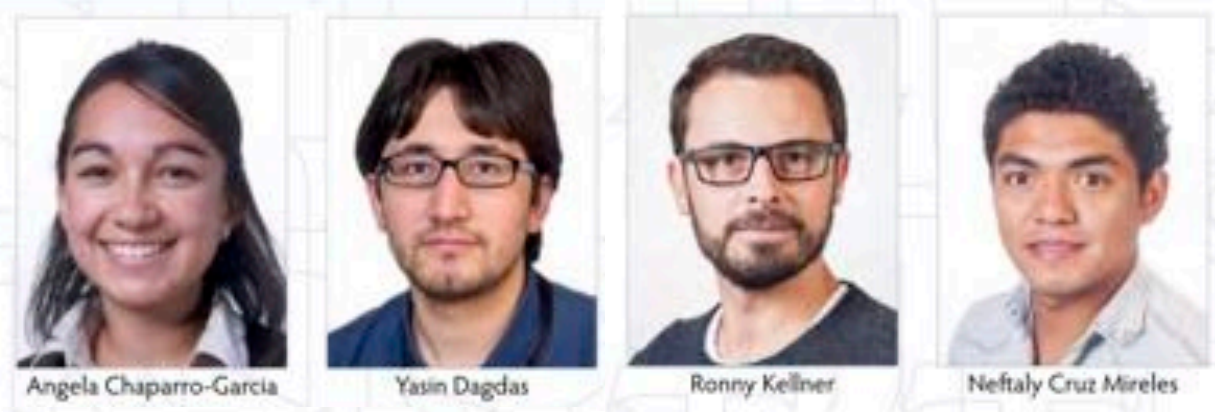
2017

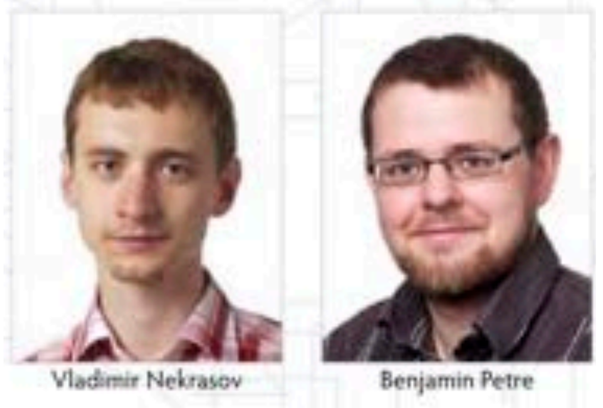
Bialas, Zess et al. bioRxiv 2017 Lessons in effector and NLR biology

Lessons in effector and NLR biology of plant-microbe systems

Aleksandra Białas[1]*, Erin K. Zess[1]*, Juan Carlos De la Concepcion[2], Marina Franceschetti[2], Helen G. Pennington[1], Kentaro Yoshida[3], Jessica L. Upson[1], Emilie Chanclud[1], Chih-Hang Wu[1], Thorsten Langner[1], Abbas Maqbool[1], Freya A. Varden[2], Lida Derevnina[1], Khaoula Belhaj[1], Koki Fujisaki[4], Hiromasa Saitoh[4,6], Ryohei Terauchi[4,5], Mark J. Banfield[2], Sophien Kamoun[1†]

@KamounLab alumni 2015/17

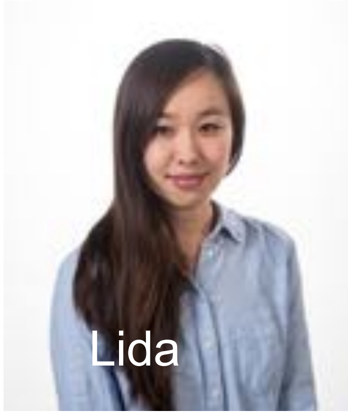





Artemis Giannakopoulou


Marina Pais

@KamounLab ~2017



GATSBY

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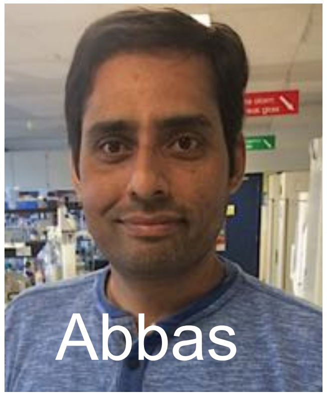
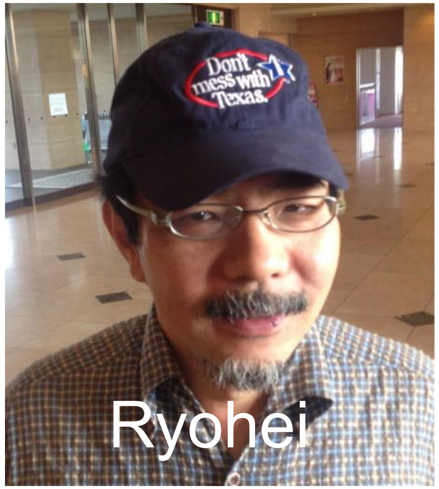
BBSRC
bioscience for the future

@MJBanfield Lab



@Terauchi Lab



Practicals – choose your session

1. Identification of effector-plant protein complexes by coIP/MS

morning: coIP (training lab)
afternoon: coIP continued, MS dataset analysis (training theatre)

Erin Zess

2. Identification of protein-protein interaction partners by Yeast-two-hybrid (Y2H)

morning: Yeast-two-hybrid Theory and experimental design
afternoon: Yeast Co-transformation and Y2H assay

Thorsten Langner

3. Agroinfiltration and virus-induced gene silencing

morning: Designing VIGS and Complementation experiments (Training theatre)
afternoon: VIGS and Agroinfiltration (Kamoun Lab)

Chih-Hang Wu

Practicals – for those who choose session 1 or 2

3rd August, 1730 - Discussion of follow-up experiments

Presentation by **Juan Carlos** on *in vitro* methods to characterize protein-protein interactions