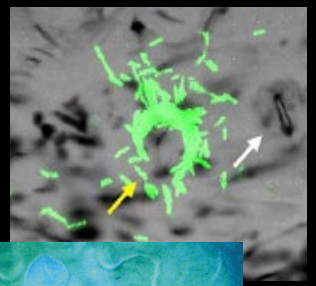
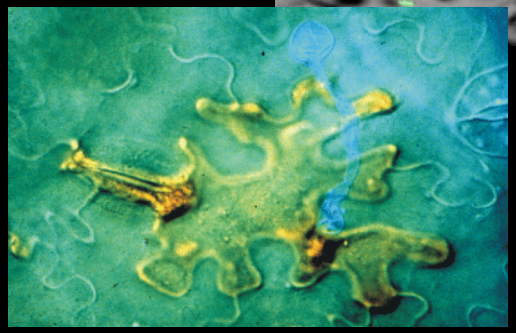
Julius-Maximilians
UNIVERSITÄT
WÜRZBURG

Molecular studies on

Plant Pathogen Interaction





Prof. Dr. Wolfgang Dröge-Laser
Julius-von-Sachs-Institute

What is defining the crop yield?

Zeus

Demeter

Novoplansky, A. and Williams, M.E. (May 31, 2013). Plant Interactions. Teaching Tools in Plant Biology: Lecture Notes.
The Plant Cell (online), doi/10.1105/tpc.113.tt0513.

Plant disease – a historical view

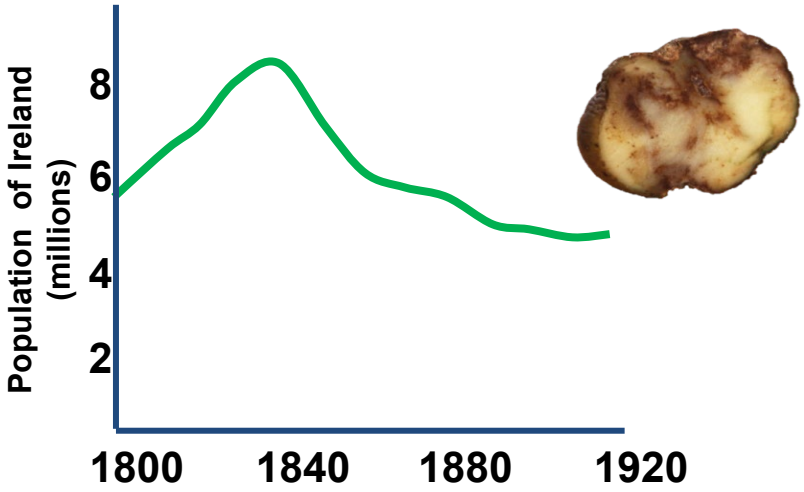
Infection of potatoe by the oomycete
Phythophthora infestans

[Ireland 1846/47]

I believe that the plants are sick because the mold is growing on them

Miles Joseph Berkeley, 1846

..many Irish people emigrate to America…

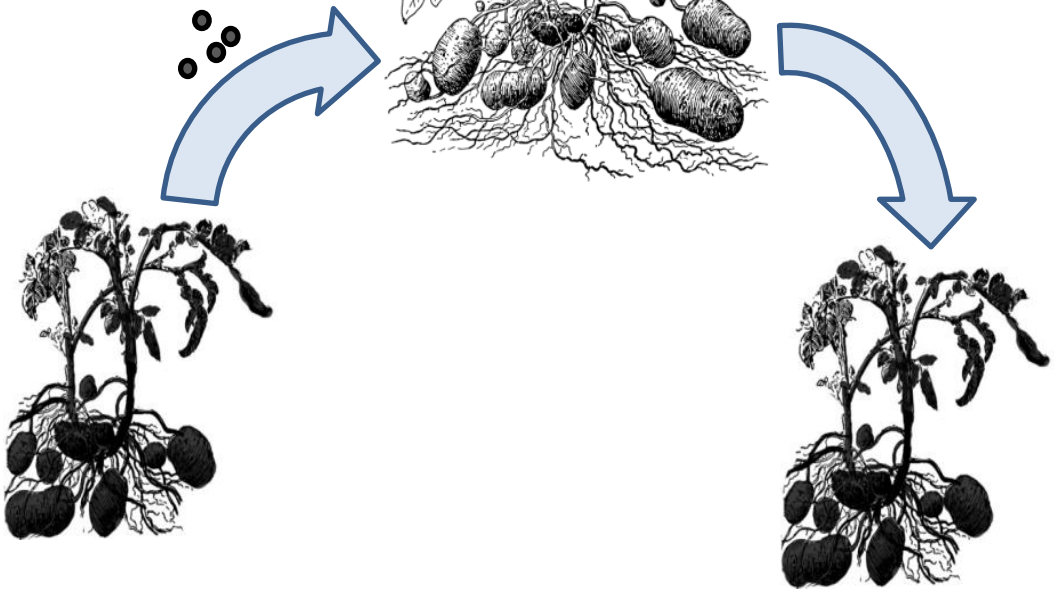


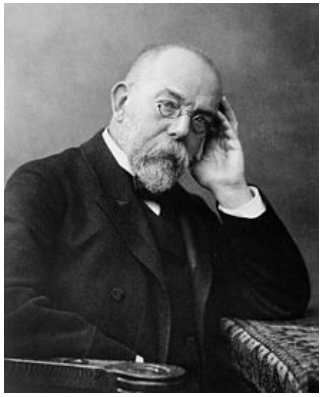
Novoplansky, A. and Williams, M.E. (May 31, 2013). Plant Interactions. Teaching Tools in Plant Biology: Lecture Notes. The Plant Cell (online), doi/10.1105/tpc.113.tt0513.

Microorganisms induce diseases

Anton de Bary, 1863

Phytophtora





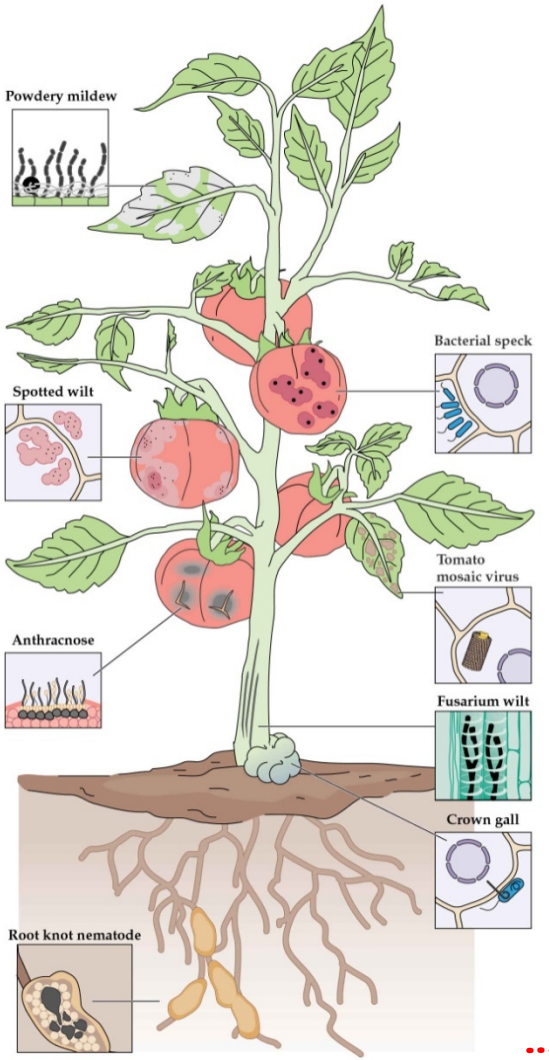
…later, in the 1880s Robert Koch and Luis Pasteur postulated that microbes are causing human deseses!

Symptoms

Novoplansky, A. and Williams, M.E. (May 31, 2013). Plant Interactions. Teaching Tools in Plant Biology: Lecture Notes. The Plant Cell (online), doi/10.1105/tpc.113.tt0513.

Interactions between a single plant species and pathogens are complex

fungi:
e.g. mold



bacteria:
e. g. Pseudomonas

Viruses:
e.g. Tomato Mosaic Virus

nematodes

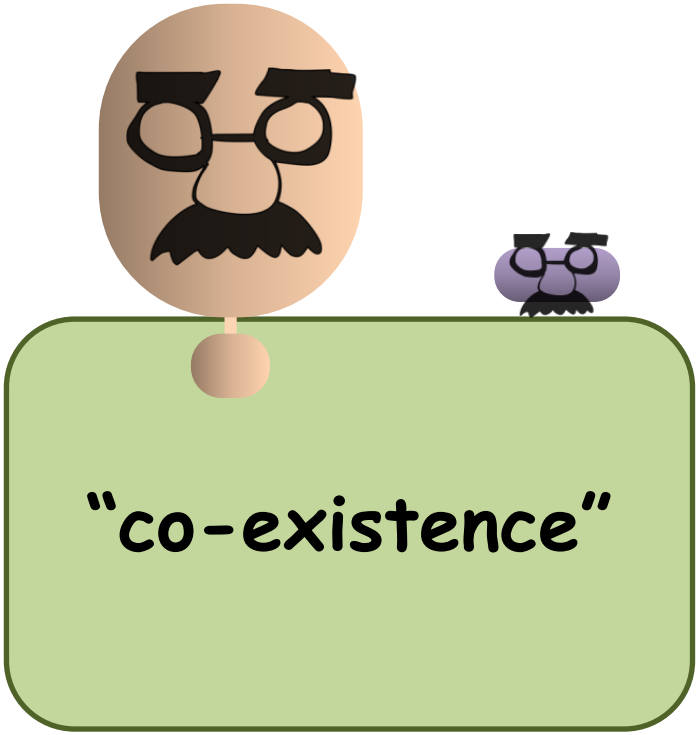
…more than 100 diseases are described for tomato!

Buchanan, Gruissem Jones: Biochemistry and Molecular Biology of Plants (2015), second edition, ASPP, Rockville, Maryland

Pathogens differ in their „Lifestyles“

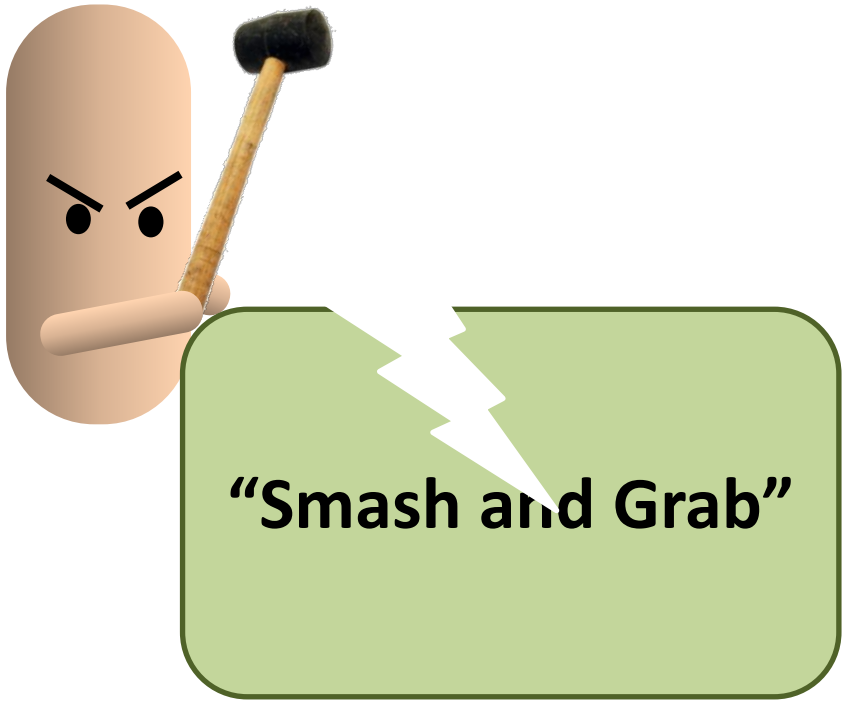
Pathogens differ in their „Lifestyles“

biotrophic



- infected plant tissues stay alive
- intimate contact between host and pathogen
- nutrients are obtained from the plant's metabolism
- limited host-range (frequently only 1 species)

necrotrophic



- host plants are actively killed to recover nutrients and to increase pathogen propagation
- by using cell-wall degrading enzymes
- by production of toxins
- frequently wide host-range

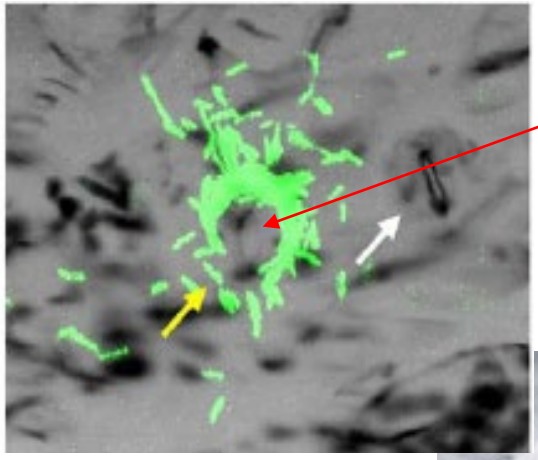
Novoplansky, A. and Williams, M.E. (May 31, 2013). Plant Interactions. Teaching Tools in Plant Biology: Lecture Notes. The Plant Cell (online), doi/10.1105/tpc.113.tt0513.

What are typical plant pathogenic microorganisms?

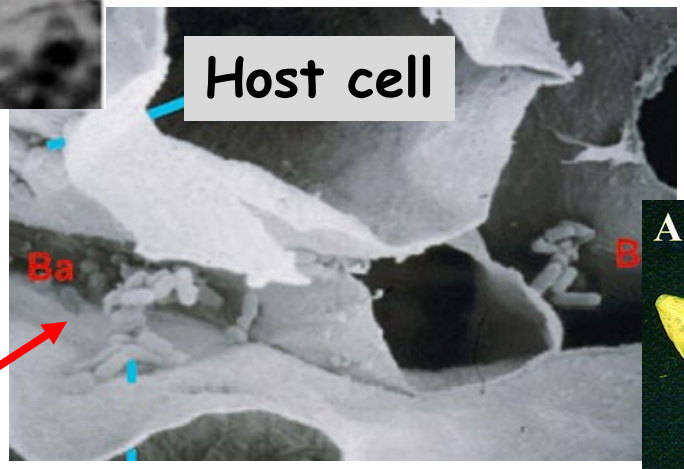
Bacteria

Pseudomonas syringae

Pseudomonas syringae propagades in the plant apoplast

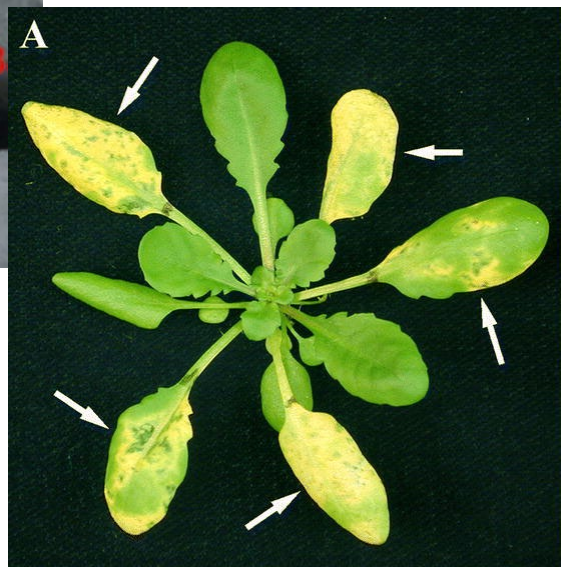


Bacteria (GFP-labled) enter the apoplast via stomata



- Bacterial propagation in the apoplast, not inside the cell
- makes use of carbohydrates derived from the plant
- (hemi)-biotrophic life-style

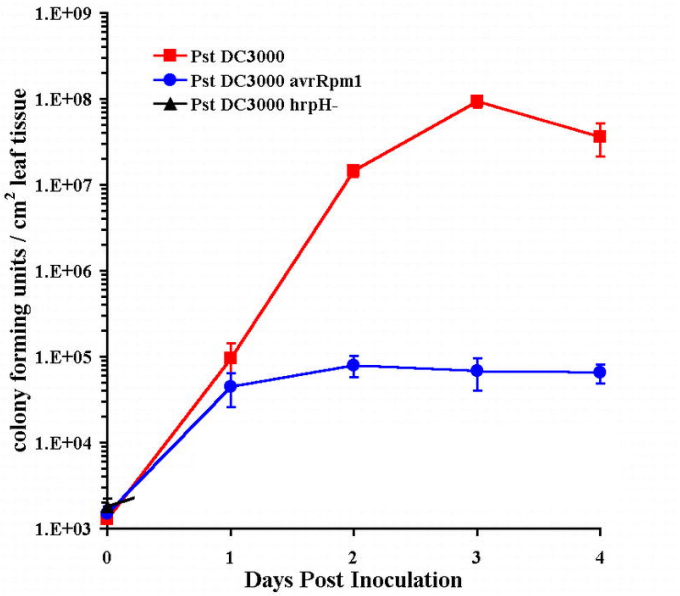
Disease symptoms
4 days
post infection (dpi)



- „water-soaked lesions"
- necroses (yellow)
- chloroses (green)

Katagiri, Thilmony, He (2002) The Arabidopsis thaliana-Pseudomonas syringae interaction, Arabidopis Book 1:e0039.

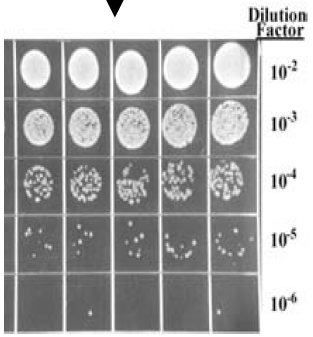
Infection under lab conditions



1-5d

grind/
extract
bacteria

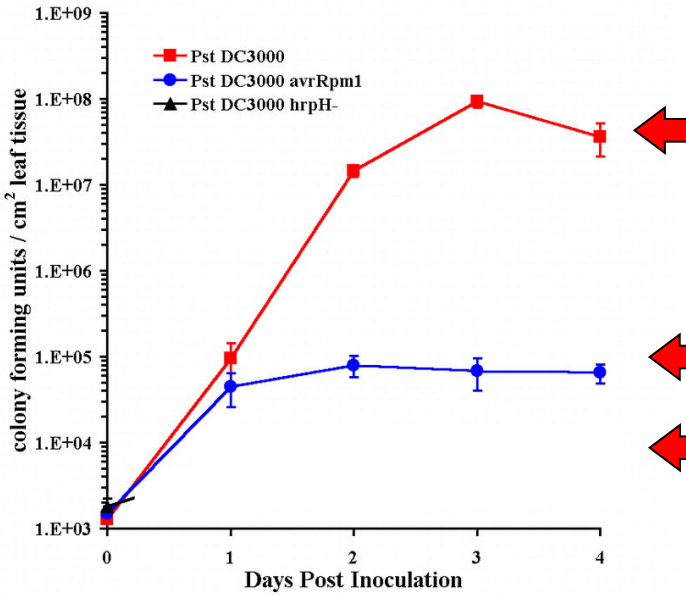
PCR
(quantify bacterial DNA)



dilution series of bactria
on agar plates
cfu: colony forming units

Katagiri, Thilmony, He (2002) The Arabidopsis thaliana-Pseudomonas syringae interaction, Arabidopis Book 1:e0039.

Quantification of the outcome of a Plant Pathogen Interaction



Rapid response?
How do recognition events differ?

"Terminology"

susceptible

rapid propagation inside the plant:
The bacterium is virulent

Interaction: "compatible"

resistant

Low propagation rate:
The bacterium is avirulent

Interaction: "incompatible"

No propagation:
The bacterium is non-pathogenic

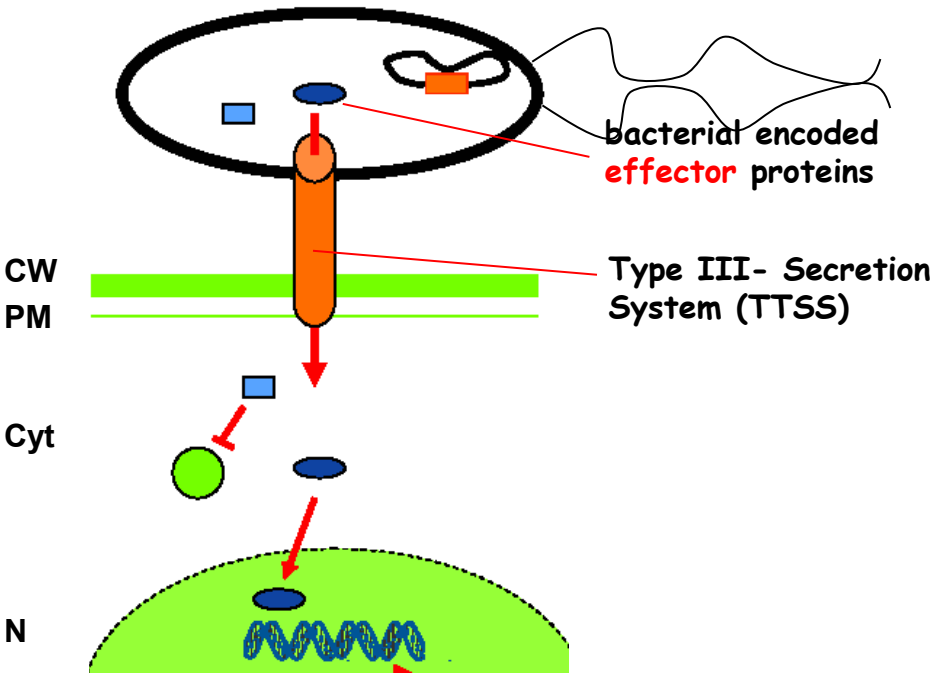
No Interaction

Which mechanisms are used by the pathogen to effectively propagate on the host plant?

„Virulence mechanisms“

Bacterial Virulence

Pseudomoas syringae



Effectors suppress
plant "targets"
important in defense

Experiment: conserved virulence mechanism

Pseudomonas aeruginosa
(broad hostrange)

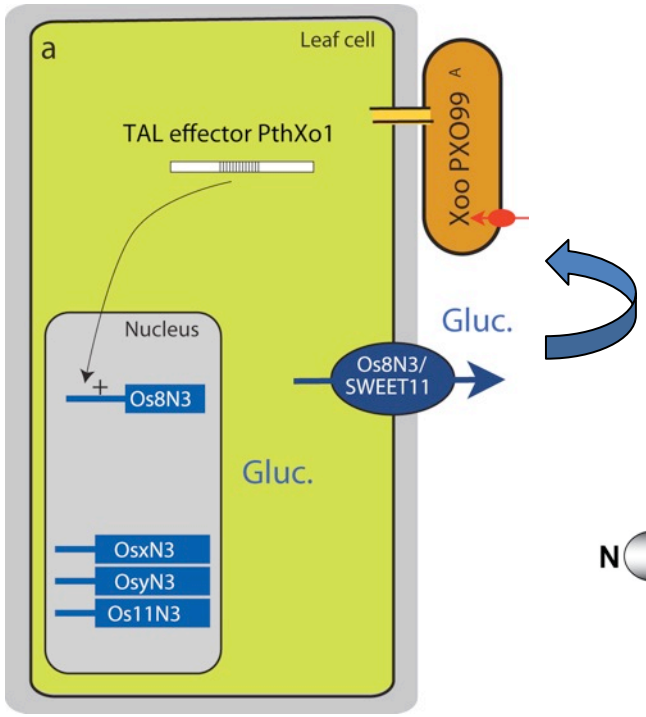
P. aeruginosa Genotype	*Arabidopsis* bacterial-titer #/ cm^2	mice (% letal)
Wildtype	1×10^7	77
hrp mutant: *plc*S	1×10^5	40
hrp mutant: *gac*A	1×10^3	0

Type III-Secretion System (TTSS)

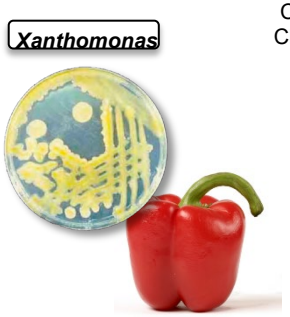
Rahme et al., Proc. Natl. Acad. Sci. USA. (1997) 94: 13245–13250

TALEs – effectors acting as transcription factors

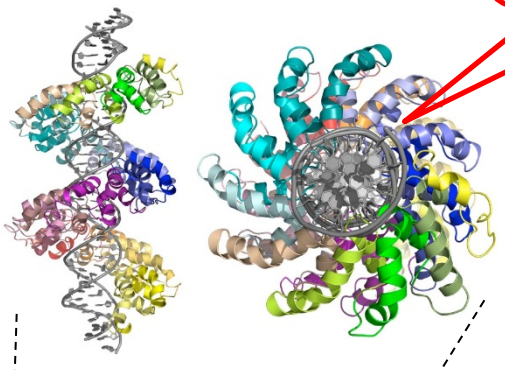
Transcription
Activator-
Like
Effectors

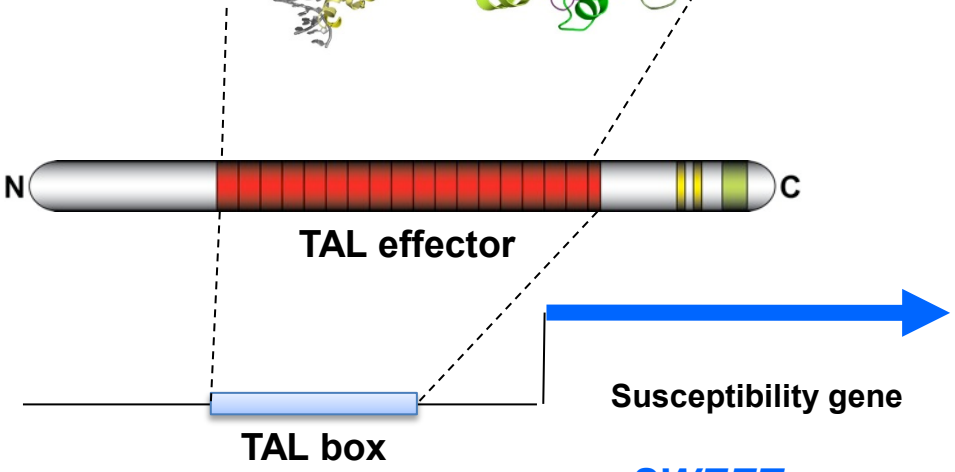


Chen *et al.* (2010) Nature
Chen *et al.* (2012) Science



… designed TALEs can be used for *gene editing* > TALENs





***SWEETs*:**
glucose & sucrose export / copper uptake

Many bacteria – many life-styles

Agrobacterium tumefaciens: ***tumors***

Erwinia carotovora: ***necrotrophci***

Gram-negative:

- Agrobakterium
- Erwinia
- Pseudomonas
- Xanthomonas
-

Gram-positive:

- Clavibacter
- Rhodococcus
- Streptomyces

Xanthomonas campestris **pv.** *campestris*

Clavibacter michiganensis: ***wilt***

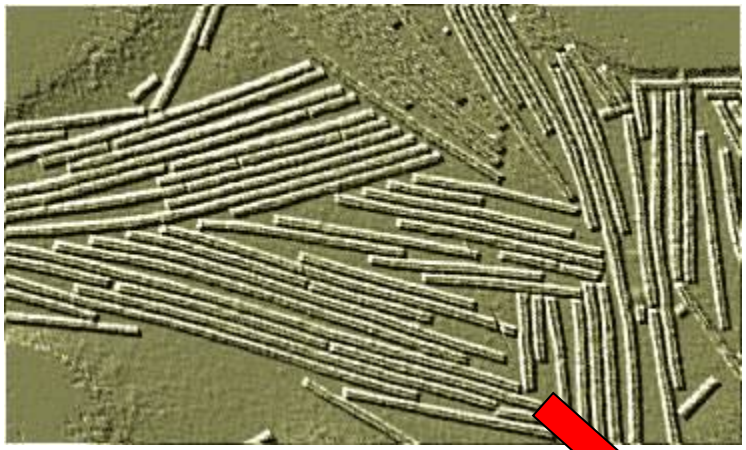
Pathogenic Organisms: Viruses

Viruses…

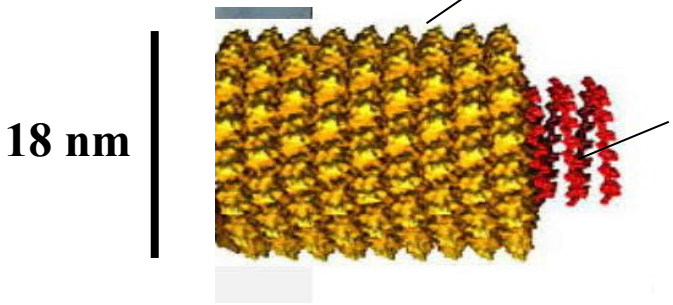
- have no own metabolism
- replicate / propagate depends on the host
- harbor genetic information (DNA or RNA)
- encode proteins (frequently:
  coat protein, replicase, movement protein)
- are very small (typically > 300 nm)

Tobacco Mosaic Virus (TMV) – a single strand RNA Virus

- first time to demonstrate disease transmission without bacterial orgnism (1890th)
- the first isolated Virus (1935)
- Nobel Prize for EM



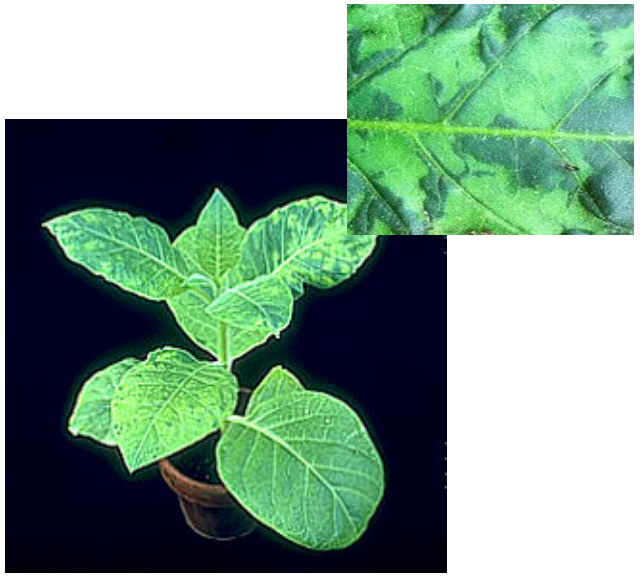
Scanning Elektron Microscopy (SEM)



http://www1.biologie.uni-hamburg.de/b-online/

Infection und disease symptoms

wounding



ssRNA (6.4 kb) encodes 3 proteins

- Replicase
- Coat Protein
- Movement Protein

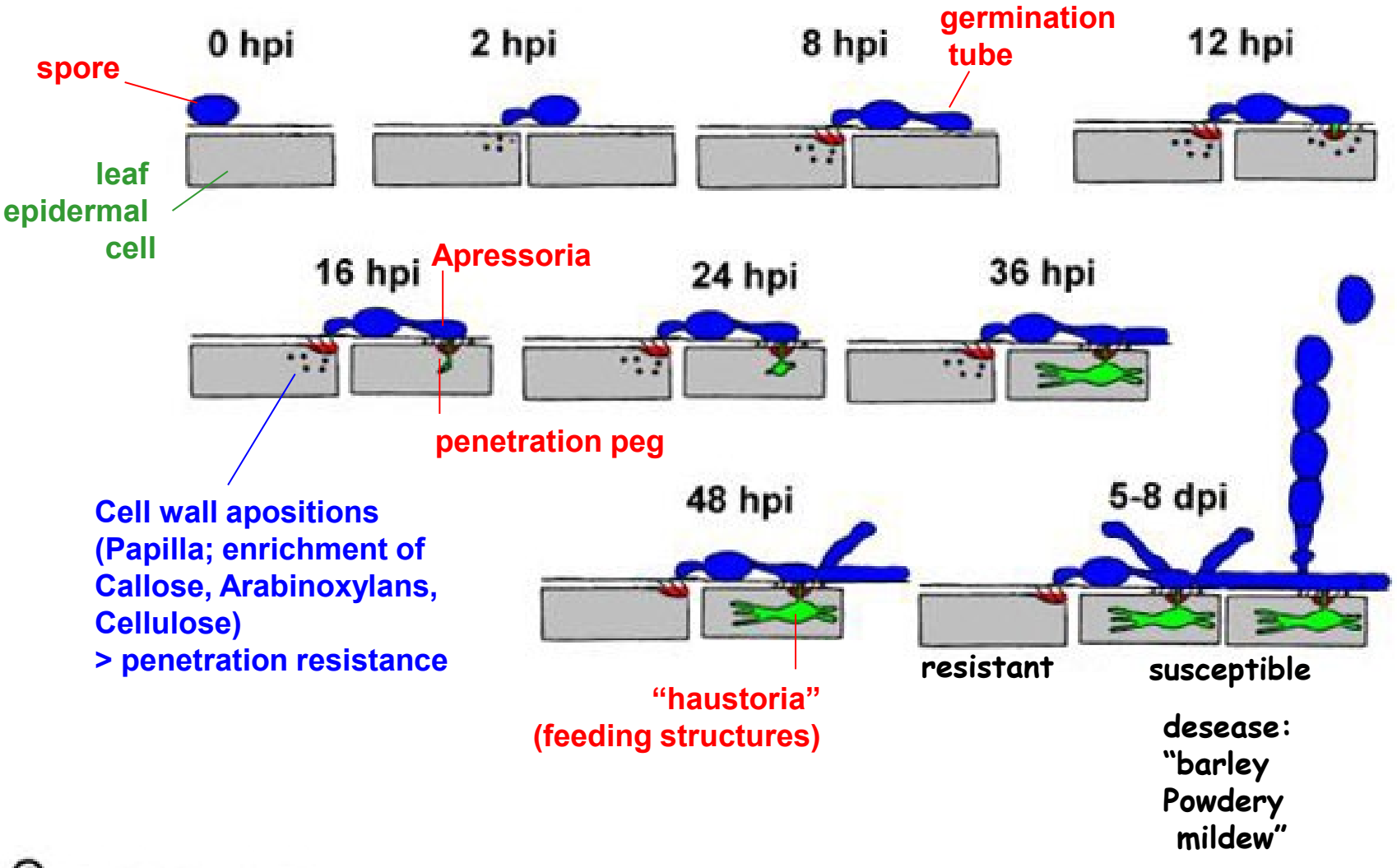
http://www1.biologie.uni-hamburg.de/b-online/

Pathogenic Organisms: Fungi

	cell-walls	spores
Oomycetes:	cellulose	zoo-spores w. flagella
Fungi:	chitin	no flagella

(Mycomycetes)

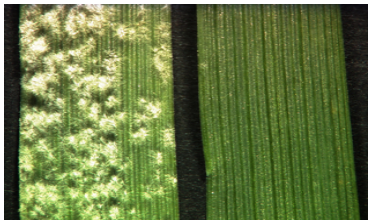
Blumeria graminis - a biotrophic fungus on barley



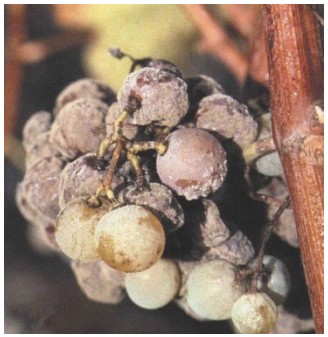
(modified)

Pathogens differ in their „Lifestyles“

biotrophic — **necrotrophic**



hemi-biotrophic



"barley powdery mildew"
Blumeria graminis

- in the beginning: biotrophic phase
  later: switch to necrotrophic life-style
- intermediate host-range

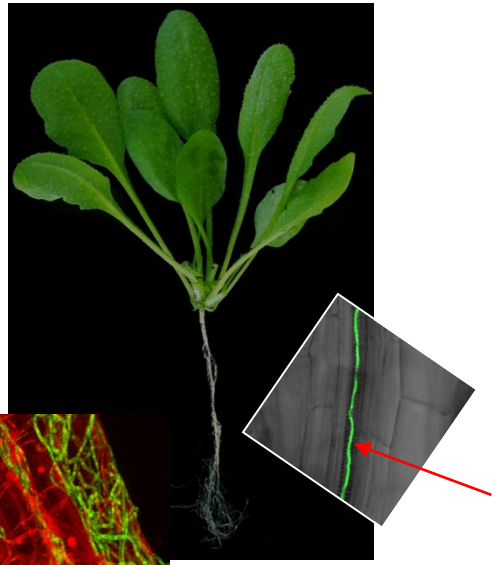
"Botrytis bunch rot"
Botrytis cinerea

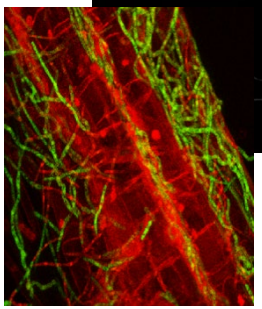
infection to sporulation
In 3d!

Oomycete *Phytophthora infestans*

Verticillium longisporum – a fungal root pathogen

- reduced growth
- chlorotic leaves
- early senescence
- early flowering



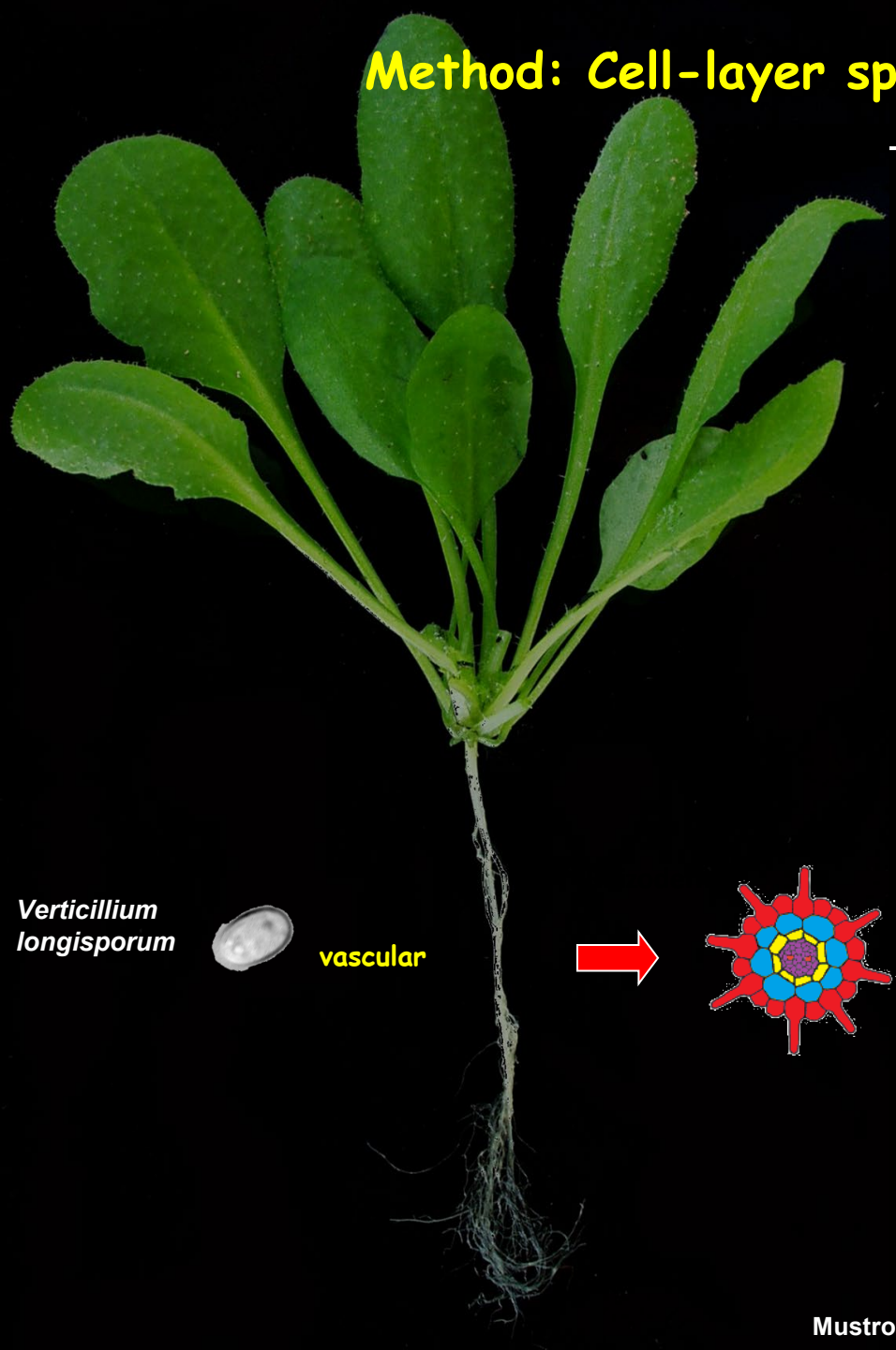


GFP-tagged
V. longisporum

„vascular" life-style:
fungal hyphae transvers the root cell-layers, enter the xylem and spores are transmitted to the foliage, where they induce damage

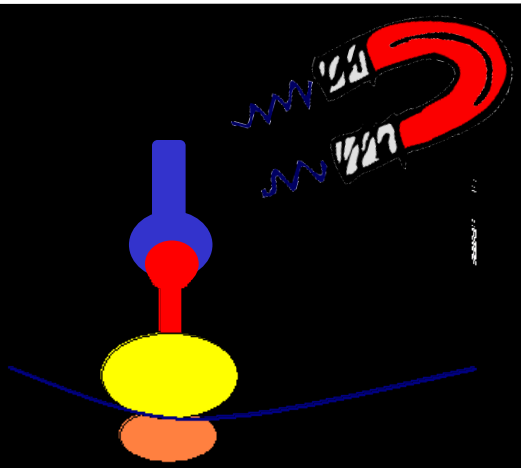
Iven et al. Mol. Plant (2012)
Fröschel. et al. MPMI (2019

Method: Cell-layer specific root translatomes



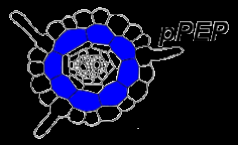
Verticillium longisporum

vascular



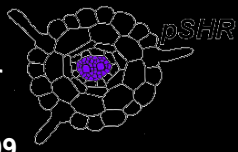
Rhizo-dermis /

Cortex



Endo-dermis

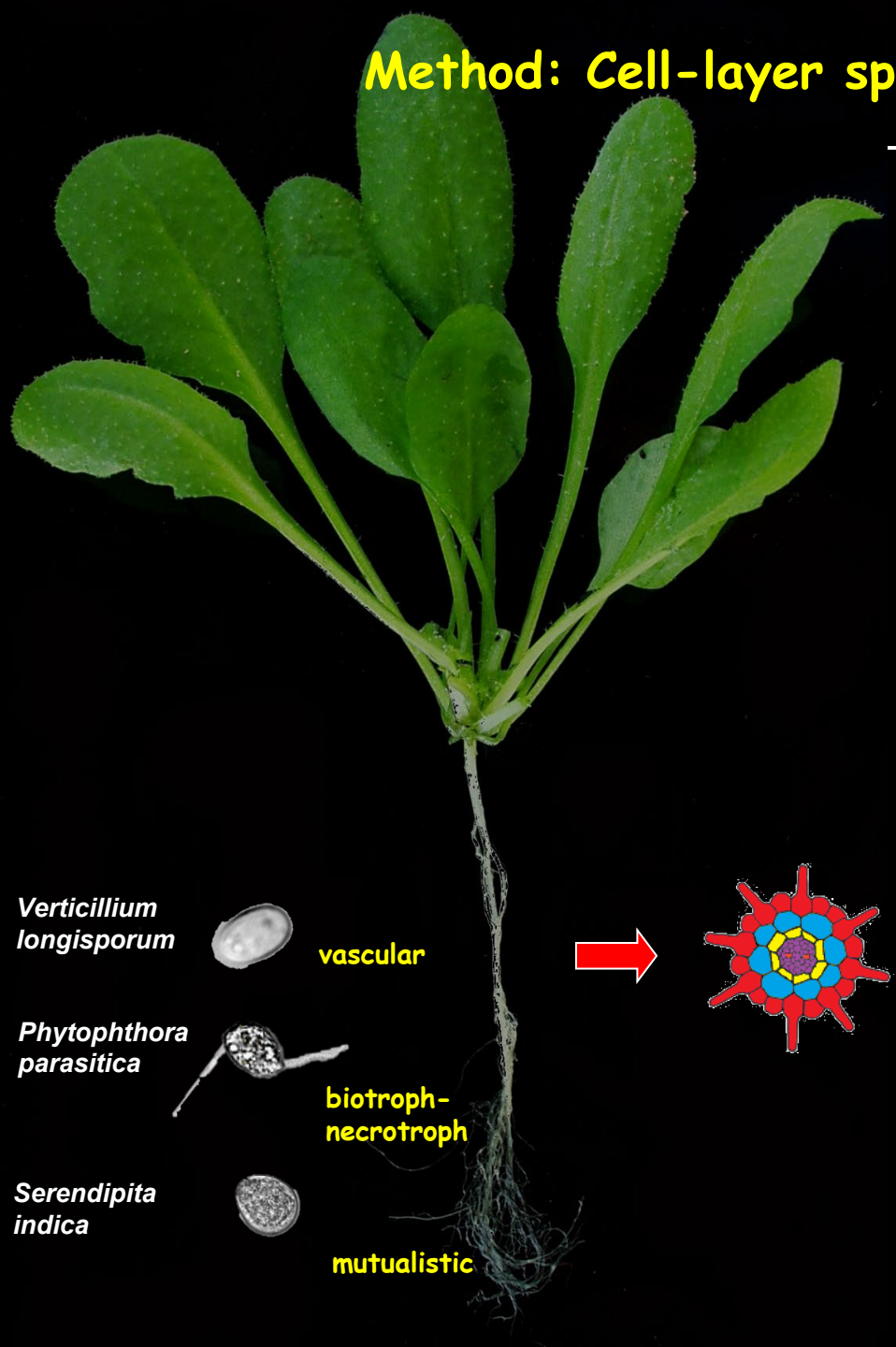
Central Cylinder



Mustroph *et al.*, PNAS 2009

Method: Cell-layer specific root translatomes

Dröge-Laser lab



Verticillium longisporum

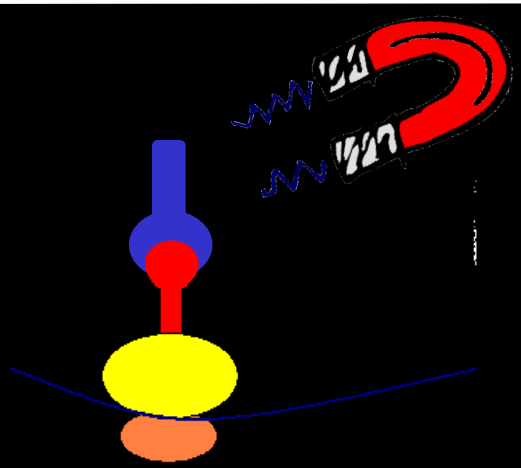
vascular

Phytophthora parasitica

biotroph-necrotroph

Serendipita indica

mutualistic



RNAseq ("Translatome")

Rhizo-dermis / pGL2

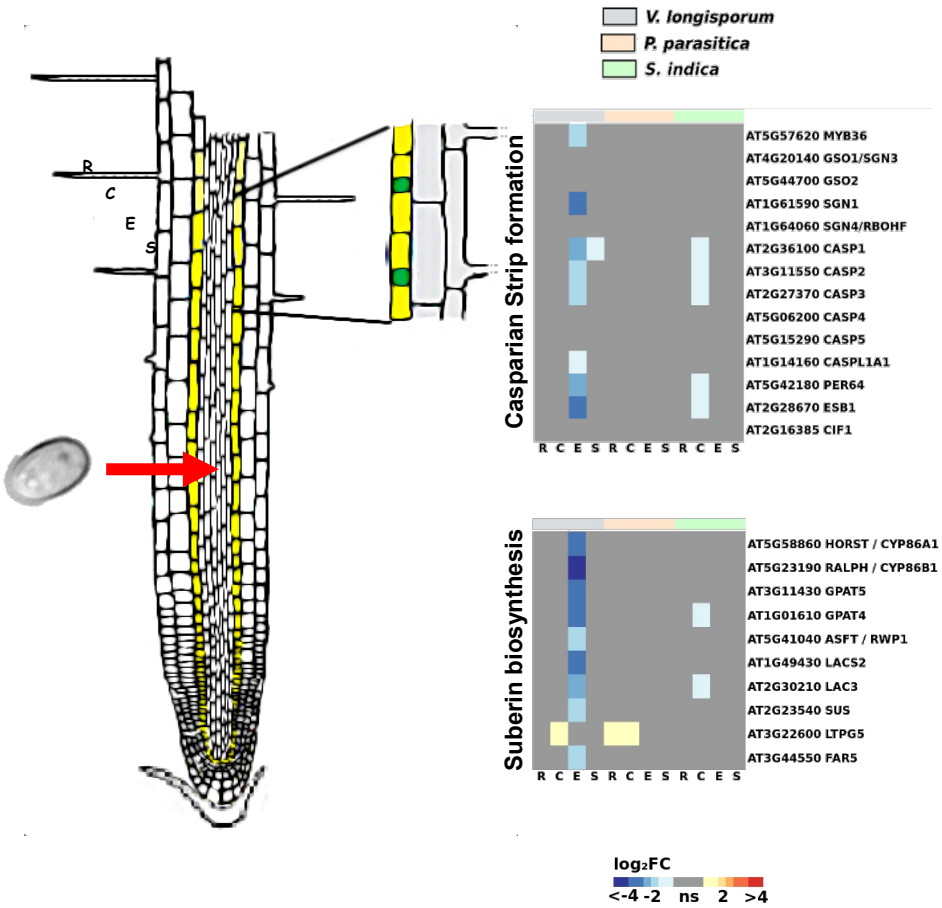
Cortex pPEP

Endo-dermis pSCR

Central Cylinder pSHR

Verticillium manipulates the endodermal barrier to proceed to the vasculature

Verticillium suppresses endodermal Genes:



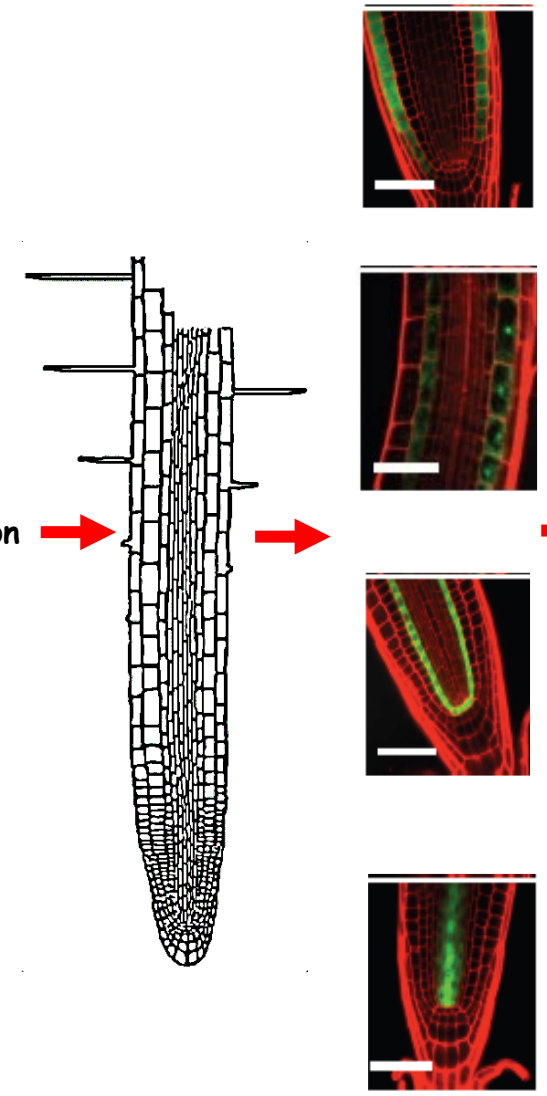
infection leads to reduced endodermal suberin:

mutants affecting endodermal barrier are more susceptible:

Fröschel et al. Cell Host Microbe (2021)

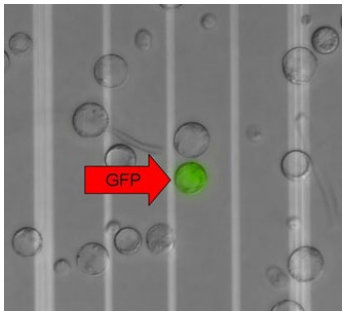
Single-cell transcriptomics: cell-layer transcriptomes upon infection

mark cell-layers by GFP



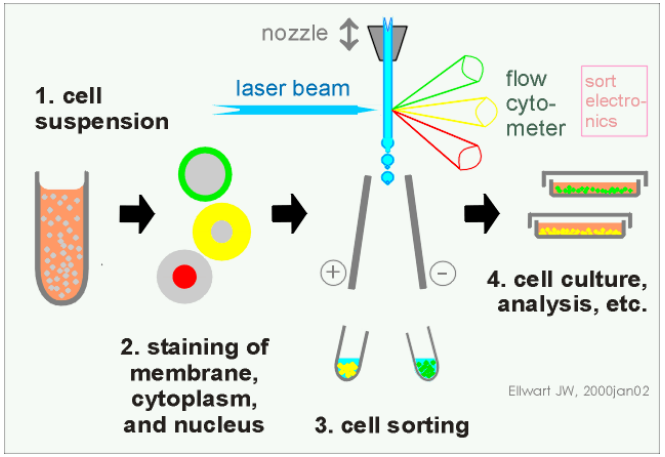
infection

digest root: protoplasts



Bargmann and Birnbaum, J.Vis. Exp. (2020)

cell sorting of protoplasts



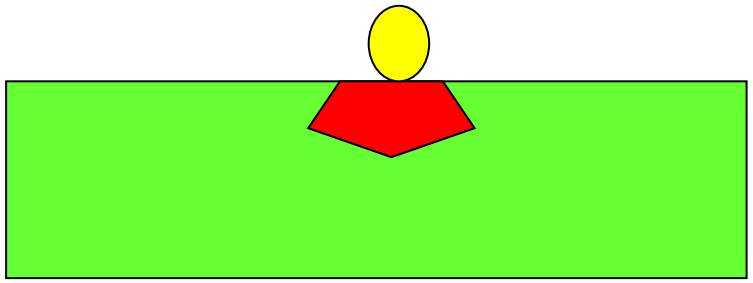
RNAseq

Rich-Griffin et al. Plant Cell (2020)

How do plants defend themselves?

Local defense

Phase 1: rapid response of single cells



1. Preformed defences

- mechanistic barriers
- chemical barriers

2. Induced defences

- Synthesis of toxic compounds („Phytoalexins")
- enhancement of the cell wall (lignin, callose)
- induction of defense genes
  (*PR* genes, *PATHOGENESIS RELATED*)
- Release/synthesis of signaling molecules / hormones
- Hypersensitive Response (HR)

Tryptophan-dependent antimicrobial compounds

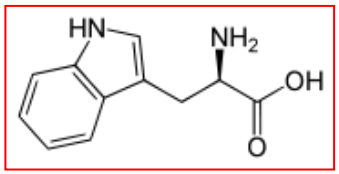
resistance assays:

- classification of symptomes
- leaf area
- fresh weight
- fungal DNA (qPCR)

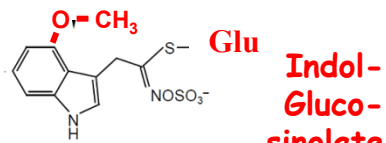
WT
(21 dpi)

cyp81f2
(21 dpi)

Chorismat
ASA1
ASB
5-Phosphoribosyl-1-PP Anthranilat
PAT1/TRP1
Indol-3-Glycerol-Phosphat
TSA
Indol
TSB1
L-Tryptophan
CYP79B2
CYP79B3
Indole-3 Acetaldoxim
CYP83B1/SUR2
CYP71A13
CYP71A12
S-Indolylmethylthiohydroximoyl-Cys
IAN
DHCA
PAD3/CYP71B15
(At3g26830)
Camalexin
I3G
CYP81F2
Indol-
Glukosinolate



antimicrobial



Indol-Gluco-sinolate

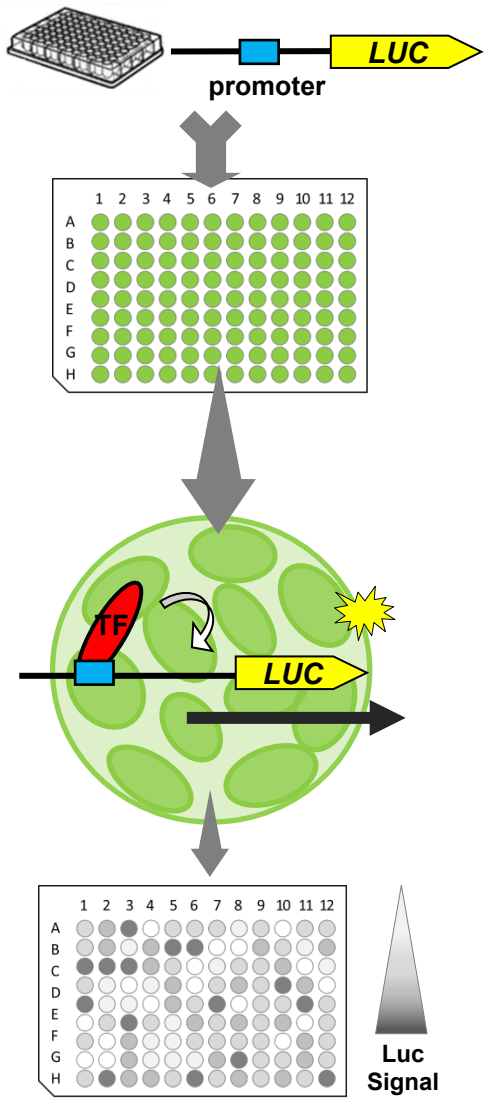
Camalexin

How to isolate TFs in Trp-derived antimicrobial compounds?

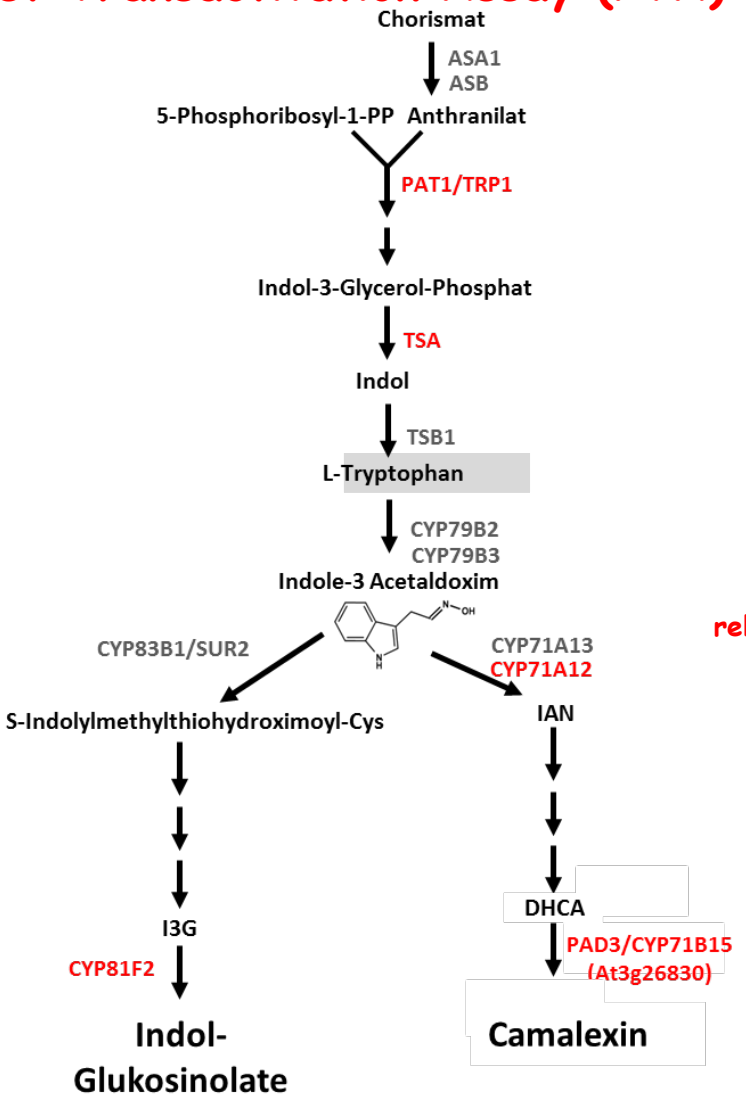
Protoplast Transactivation Assay (PTA)

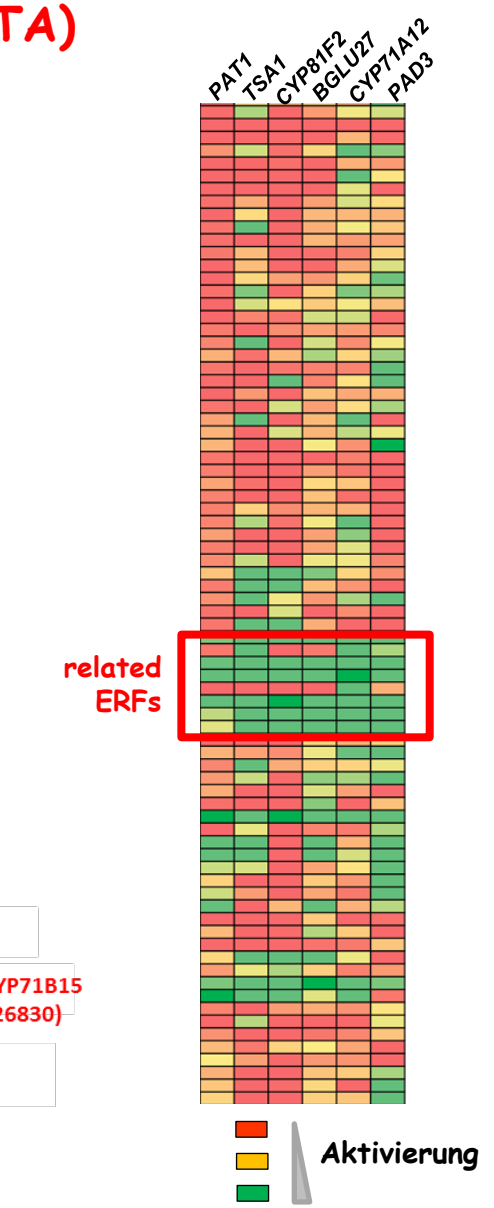
organised TF Expression Collection

(ca, 1500 TFs)



Wehner et al., Plant J. (2011)



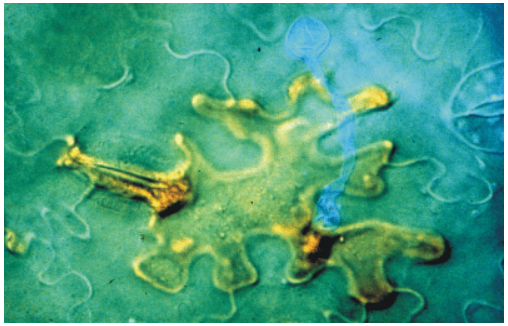


Hypersensitive Response (HR)

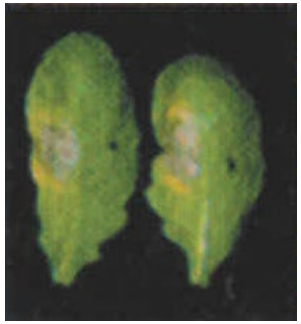
Local prgrammed cell death of infected cells:

- rapid response (*Pseudomonas syringae*: 12-24 h)
- bloocking the propagation of **biotrophic** pathogens
- restricting mutritint supply
- protecting the other parts of the plant

Pilz:
Phytophtora infestans



Bakterium:
Pseudomonas syringae

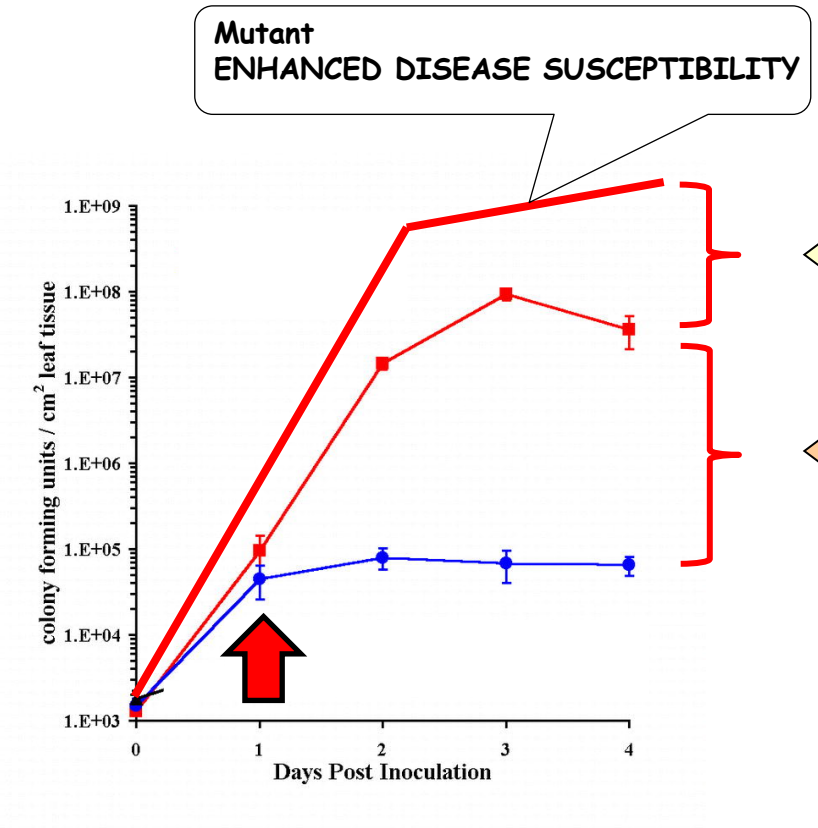


Virus:
TMV

…not effective against necrotrophic pathogene !

How are pathogens recognized ?

There are two levels of plant defence

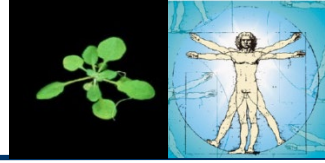


2 unspecific „basic resistance"

1 specific

early recognition event

Pathogen defence relies on two Strategies:



„innate Immunity“
Basal resistance
non-specific, non-adaptive

PAMP-triggered Immunity (PTI)

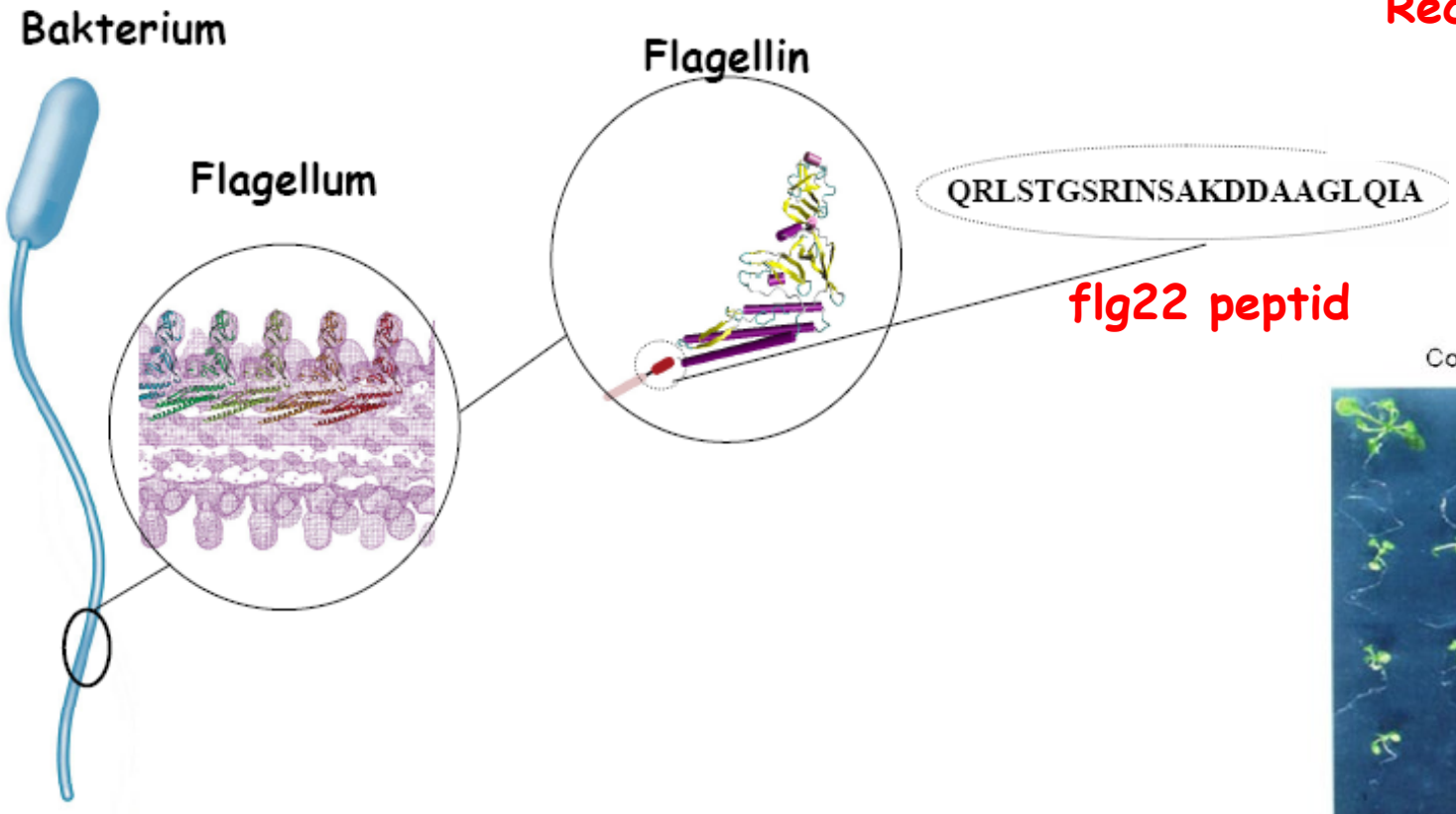
„acquired immunity“
specific

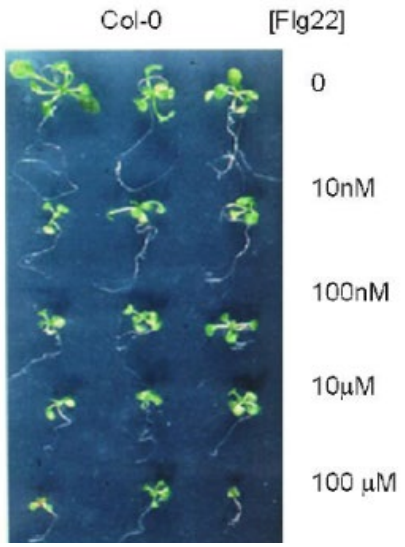
Effector-triggered Immnunity (ETI)

Basal resistance:
PAMP-triggered Immunity (PTI)

Basic resistance is based on general elicitors

How to isolate a Receptor?

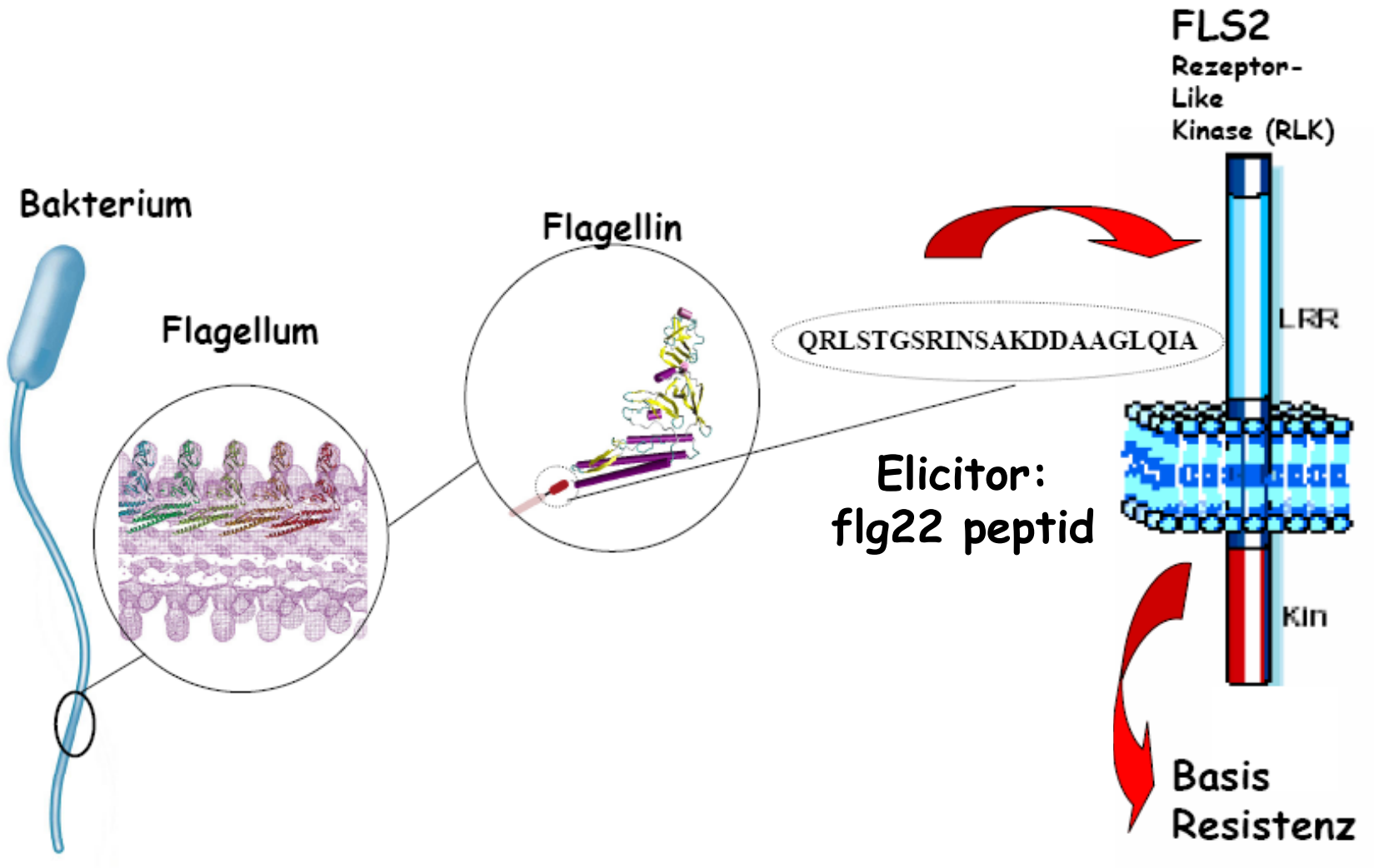




https://molbio.mgh.harvard.edu/sheenweb/pathogen.html

(Lourdes Gómez Gómez & Thomas Boller, Mol. Cell 5, 1003-1011, 2000)

Basic resistance is based on general elicitors

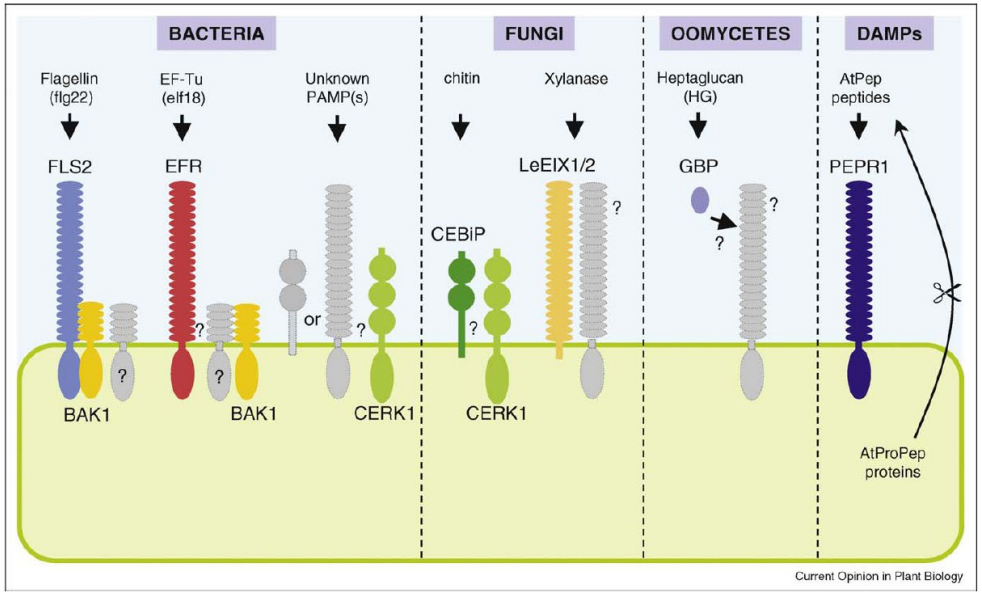


https://molbio.mgh.harvard.edu/sheenweb/pathogen.html

PAMP-triggered immunity

PAMPs („Pathogen Associated Molecular Patterns")
Or
MAMPs („Microorganism Associated Molecular Patterns")
(..non pathogenic microbes might also be recognized!)

- non-self recognition
- broad recognition based on conserved structures, frequently found
- PAMPs are essential for microorganisms



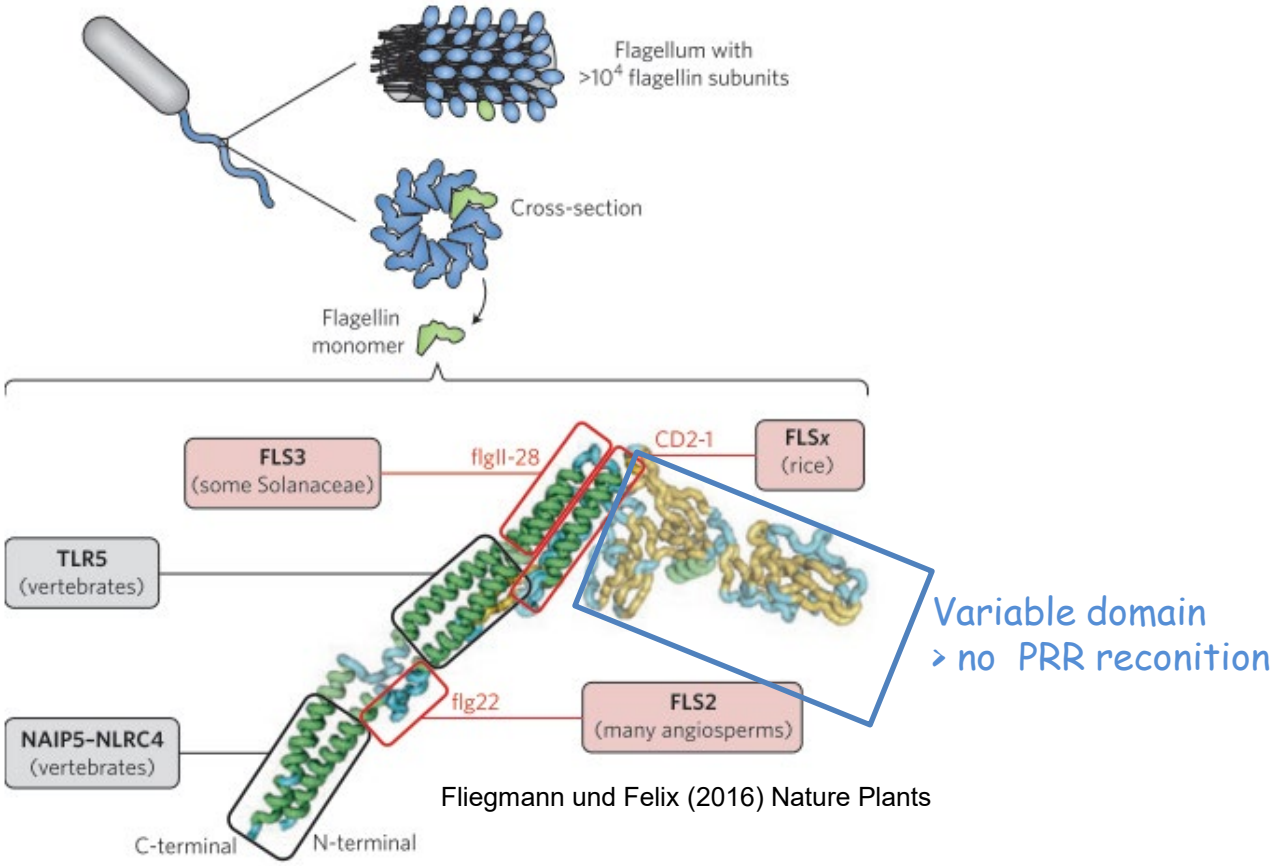
Pattern Recognition Receptors (PRRs)

recognize PAMPs

Advantages:

- stable resistance
- only a limited number of receptors is required

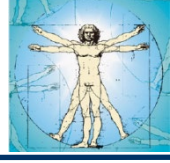
M/PAMP recognition in plants and animals



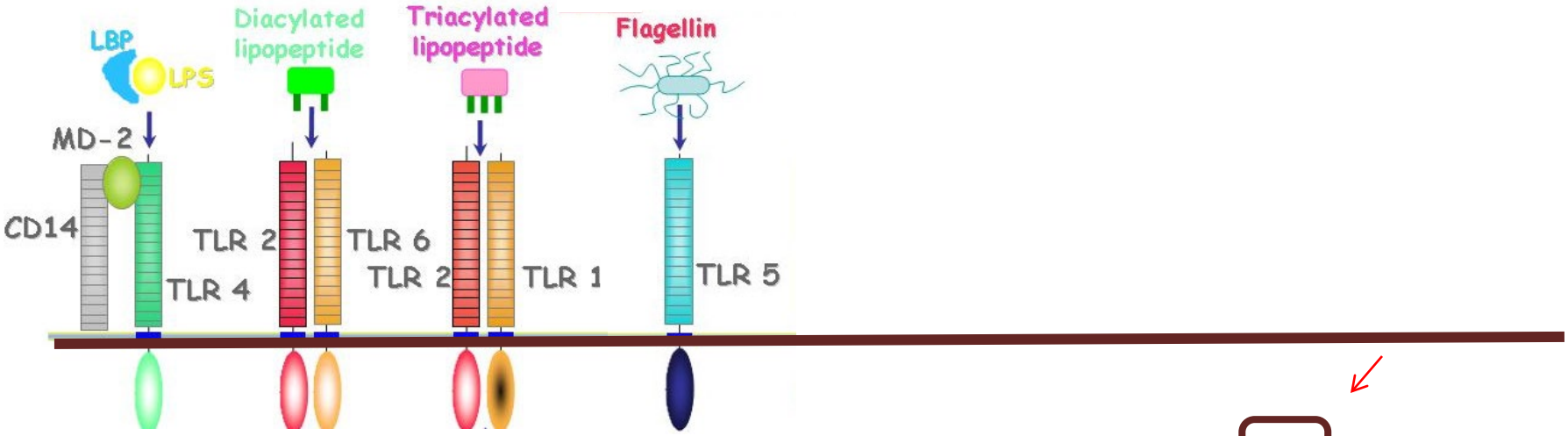
Fliegmann und Felix (2016) Nature Plants

Human (TLR5) and plant receptors (FLS2) recognize different flagellin domains
PRR don't show homology
convergent evolution

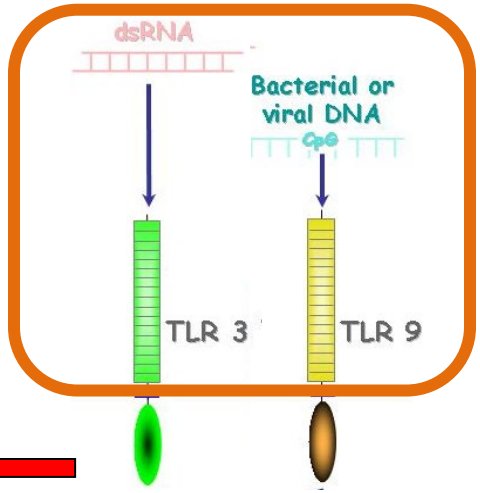
PAMP recognition in animals: Toll-like Receptors



Surface receptors:

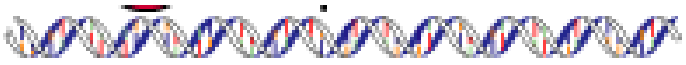


Endosom

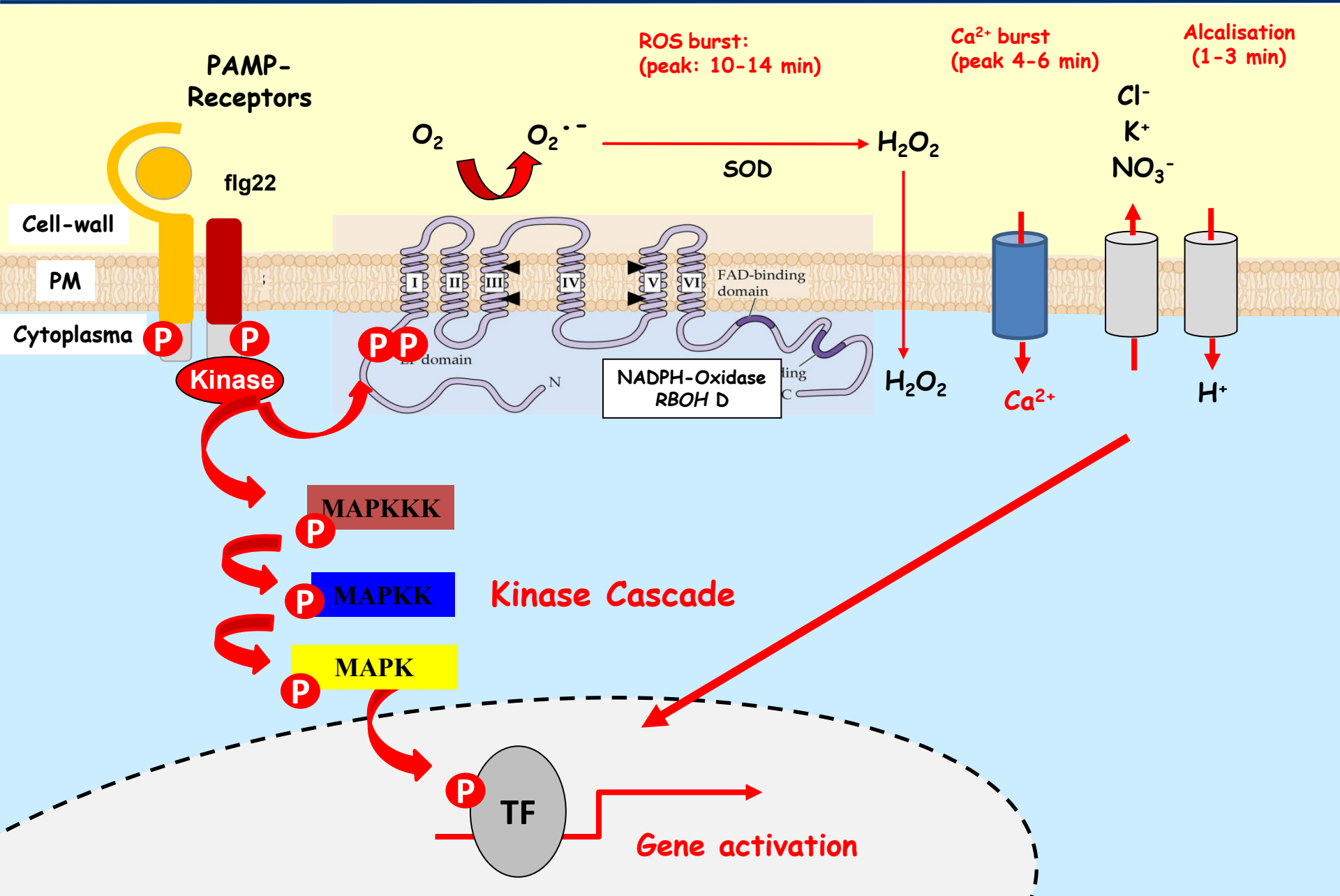


intracellular receptors e.g. viruses

Gene Expression:

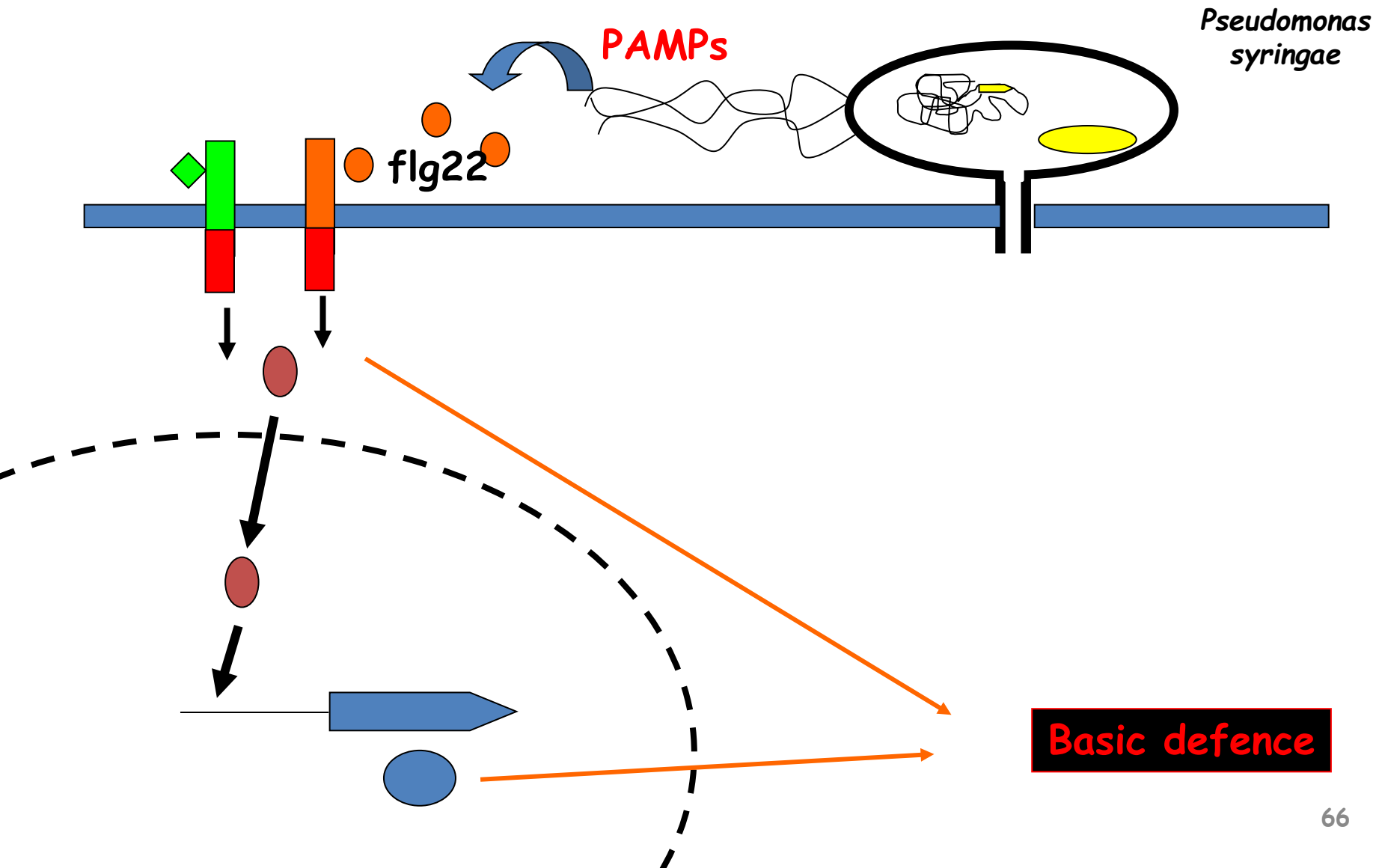


PAMP-triggered signalling

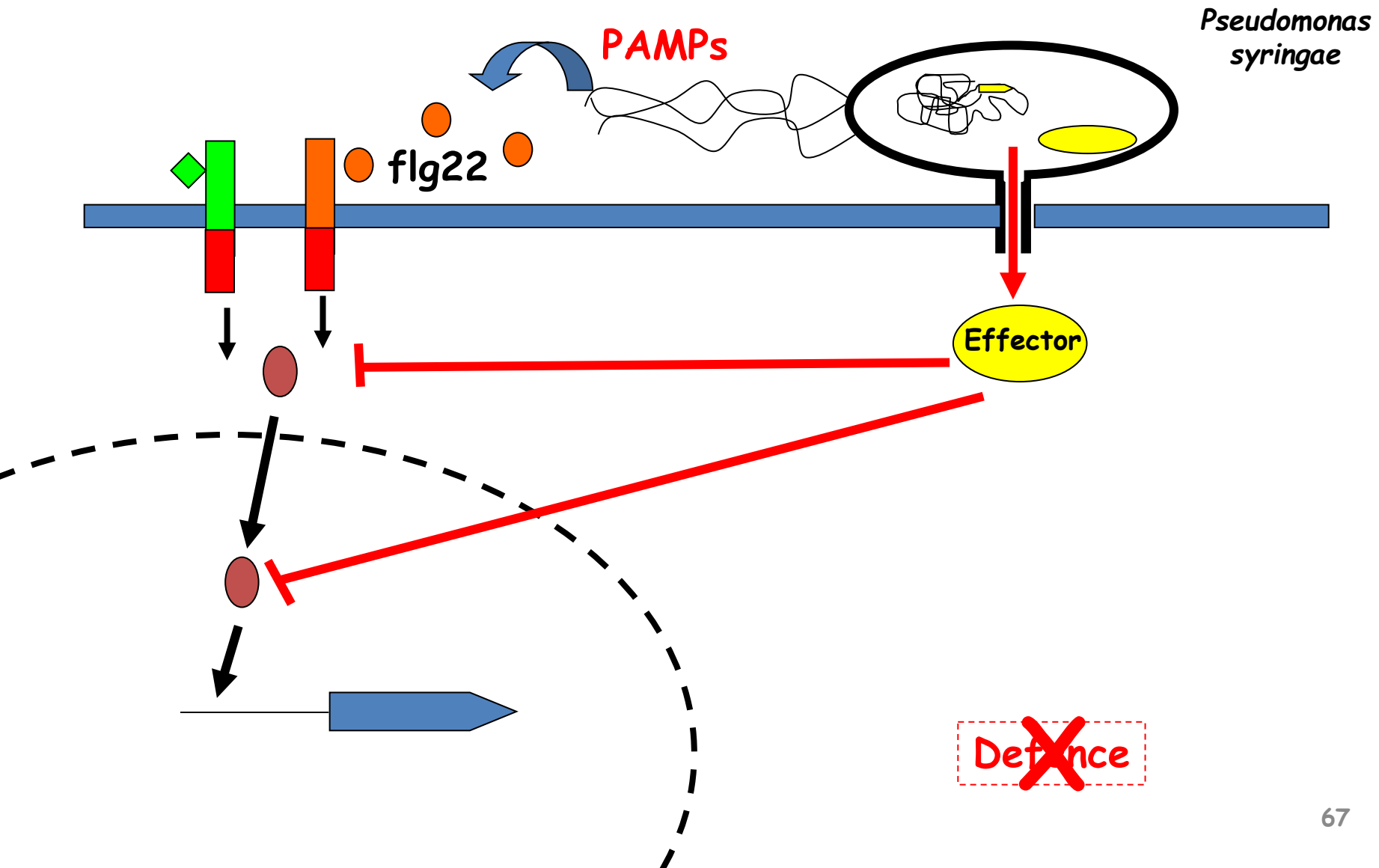


How can a pathogen overcome PTI?

PAMP-triggered Immunity (PTI)



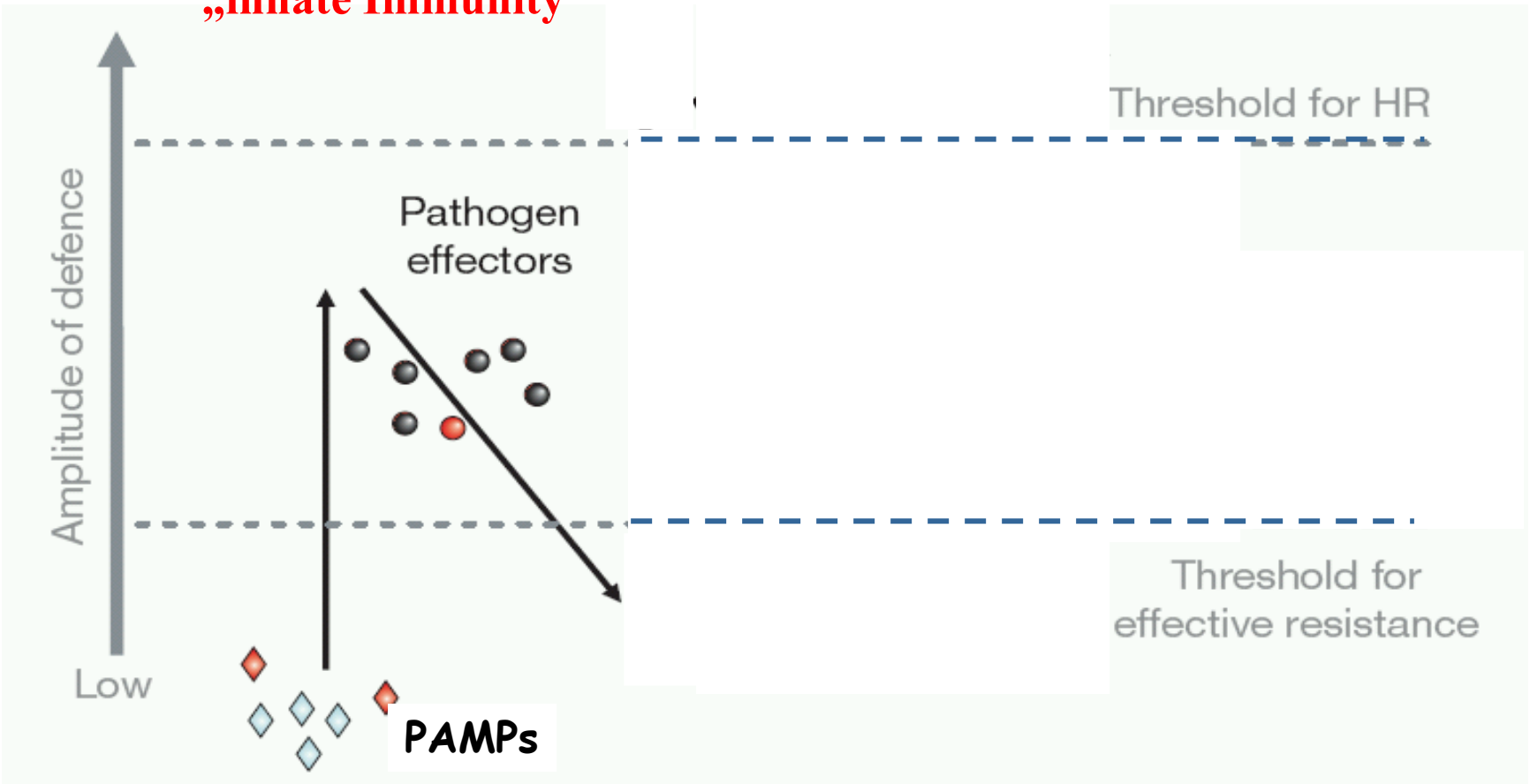
Pathogen effectors suppress Basic Resistance: „Effector-triggered Susceptibility"



„Zickzack-Model" - Co-Evolution in Plant-Pathogen-Interactions

„PAMP-triggered Immunity"

„innate Immunity"

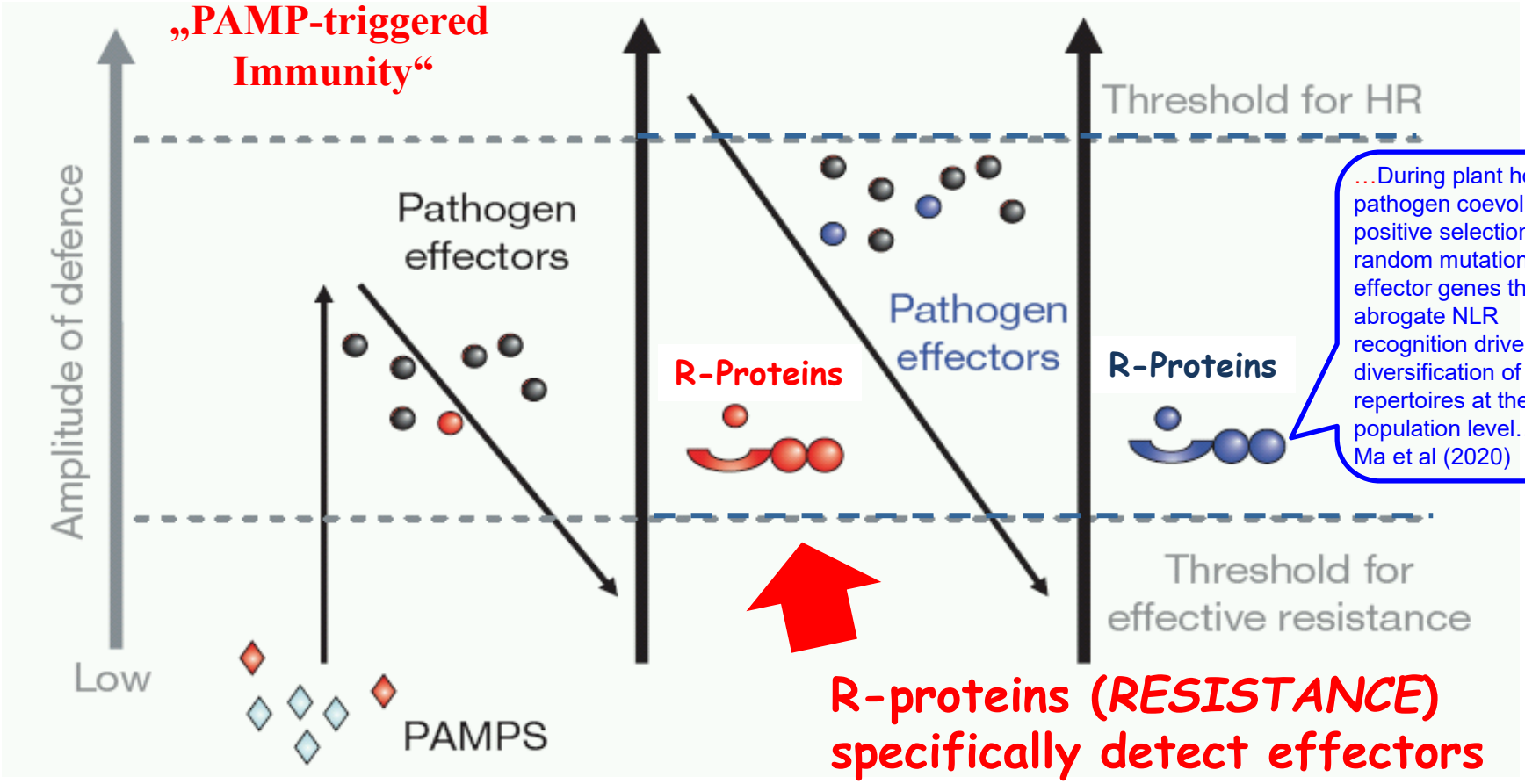


„Effector-triggered Susceptibility"

Dangl and Jones, Nature (2006)

„Zickzack-Model" – Co-Evolution in Plant-Pathogen-Interactions

„Effector triggered Immunity"



„Effector-triggered Susceptibility"

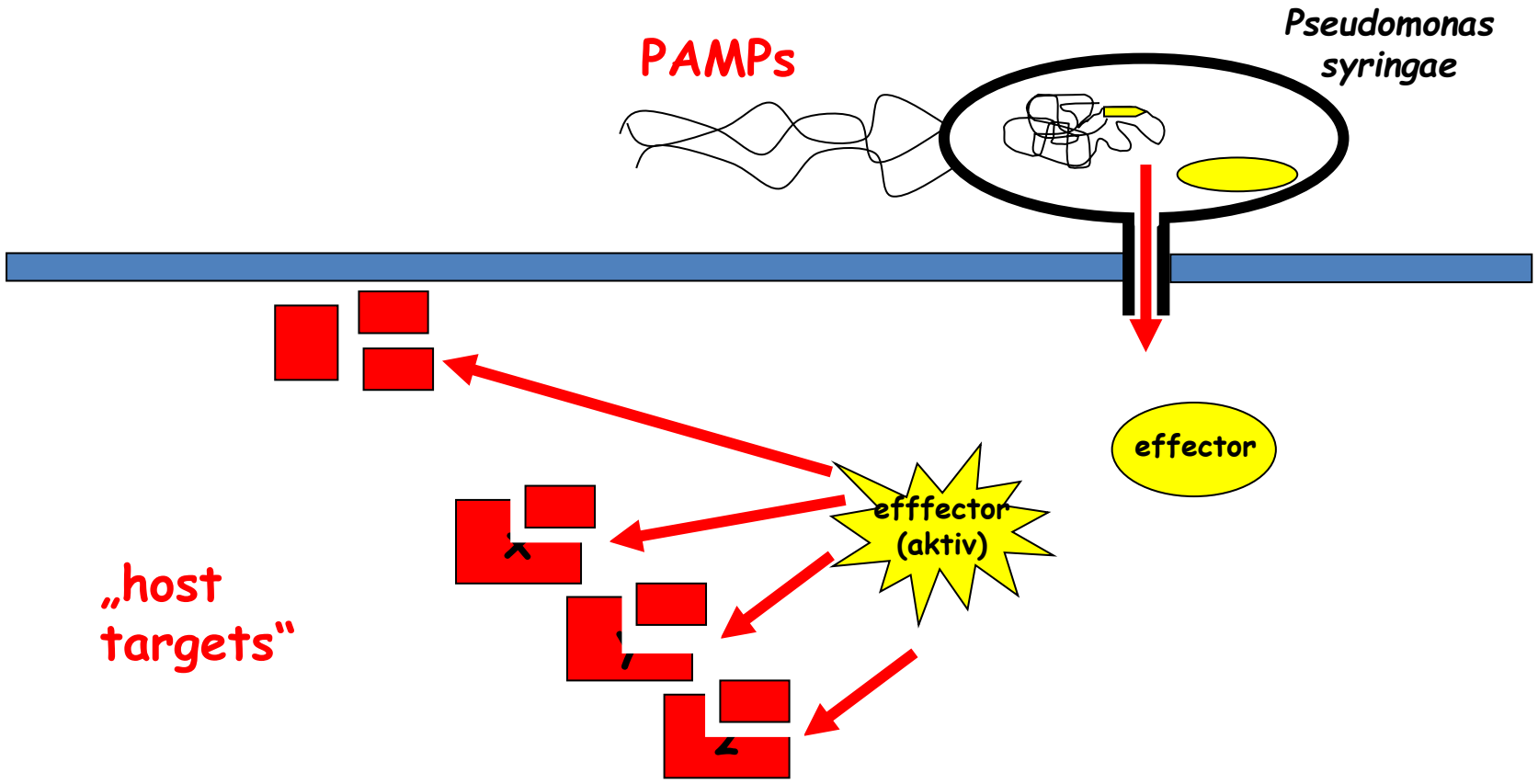
Dangl and Jones, Nature (2006)

How are effectors recognized?

The second layer of defense:

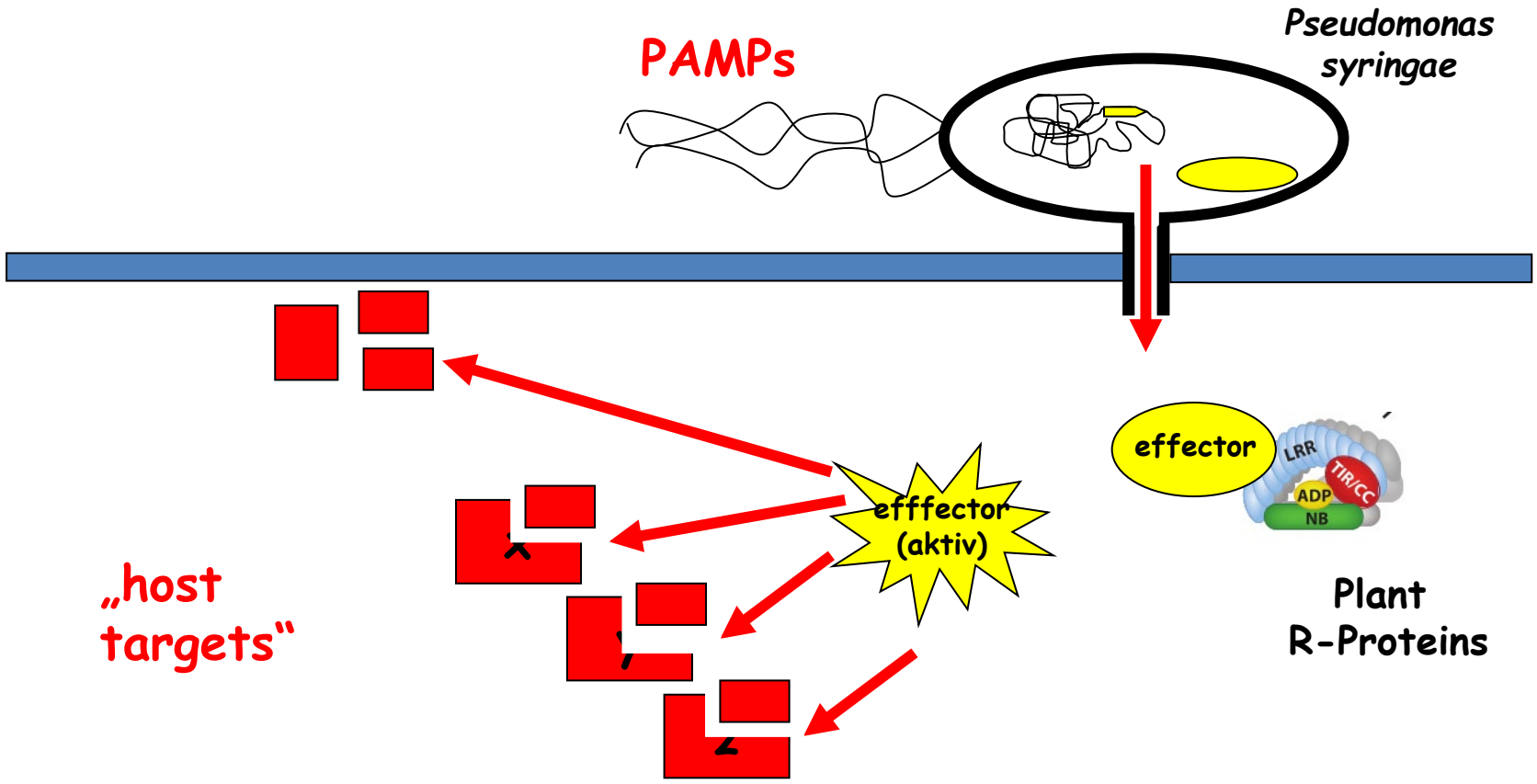
Effector-Triggered Immunity (ETI)

Effectors "attack" host targets and suppress plant defenses

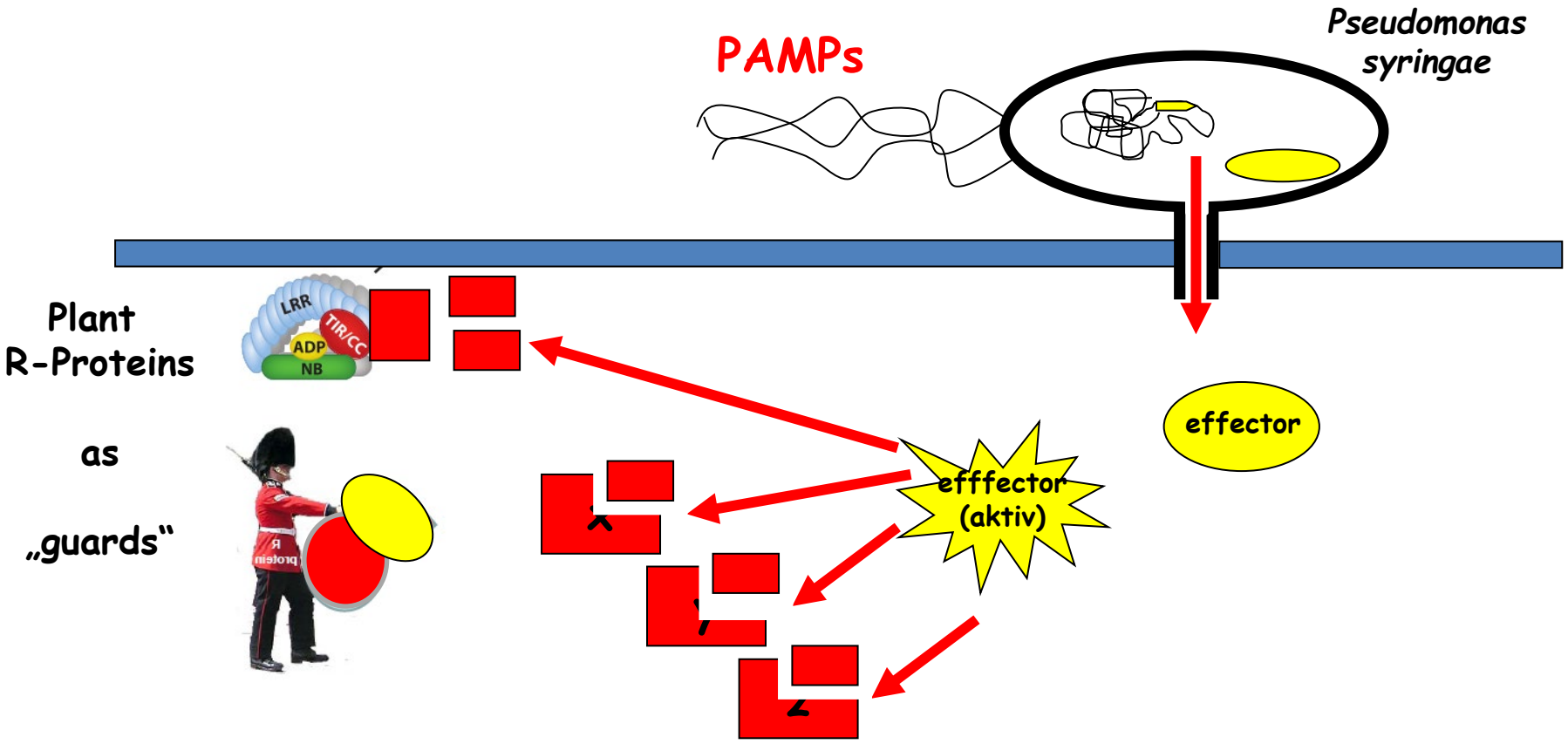


Do R-Proteins directly recognize effectors?

...sometimes

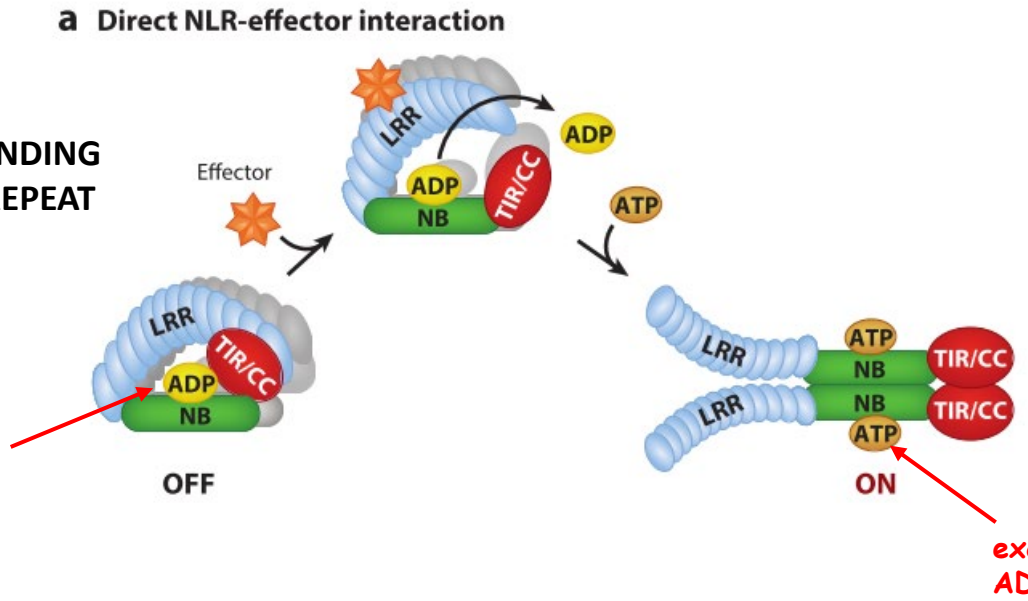


The "guard"-Hypothesis: R-proteins guard integrity of host targets



How are R-Proteins/NLRs activated?

NUCLEOTIDE-BINDING LEUCINE-RICH REPEAT RECEPTORS (*NLR*).



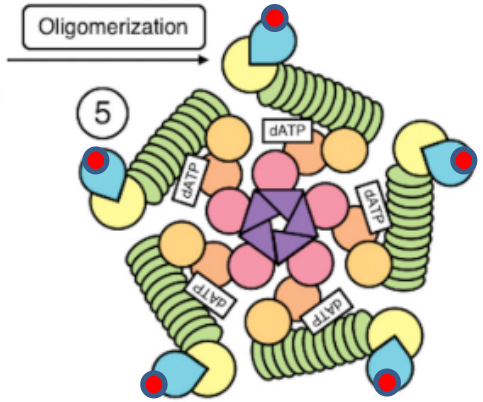
NLR-Proteins share **Sensor** and **Signaling** functions

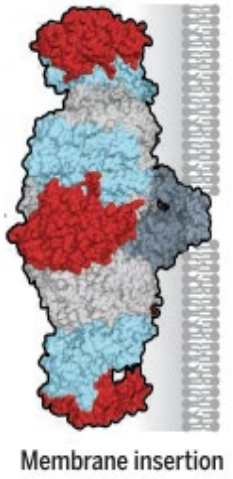
Cui, Tsuda, Parker Annual Rev. Plant Biol. (2015)

NLR activation is linked to hypersensitive cell death

Plant:

signaling by cooperative assembly formation (SCAF)

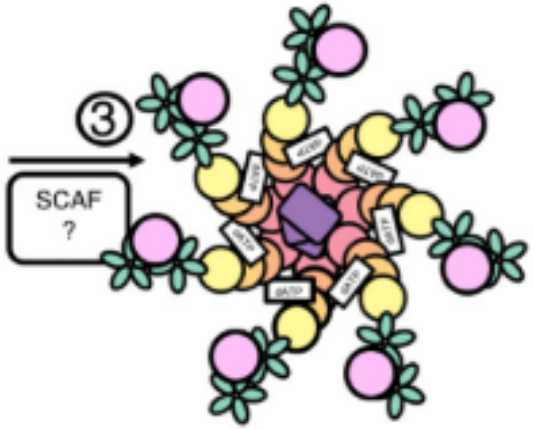


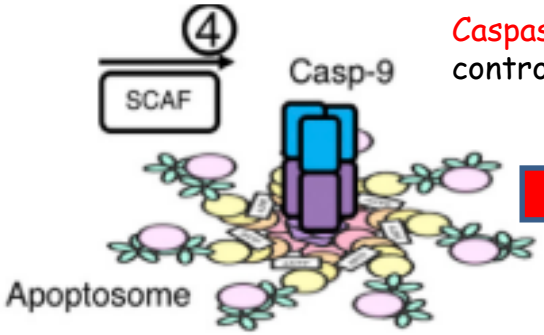


HR
Hypersensitve cell-death

aninmal: APAF1

Cytochorme C





Caspasen control Apoptosis

Apoptosis

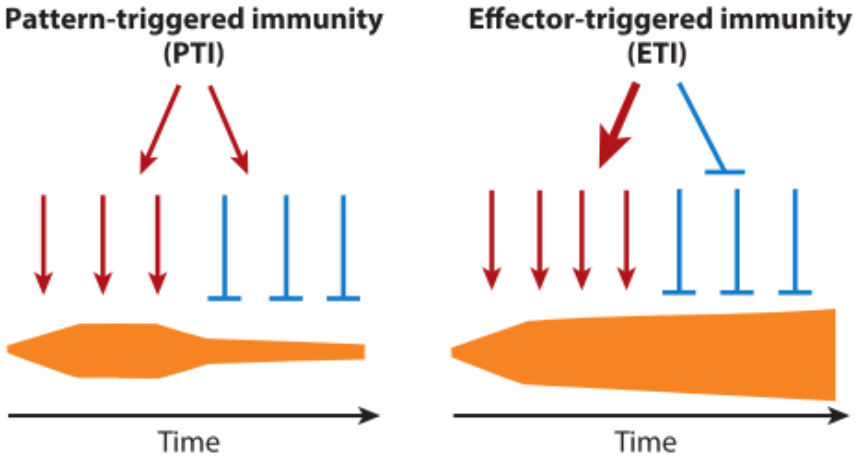
Wang et al. Sceince (2019)
Dangl and Jones Sceince (2019)
Burdett et al. Cell Host Micorbe (2020)

Pathogen defence relies on two Strategies:

PAMP-triggered Immunity (PTI)
„innate Immunity“
non-specific

Effector-triggered Immnunity (ETI)
„acquired immunity“
specific

systems are highly connected



- unspecific (based on PAMPs)
- rapid but limited in strength

- highly specific
- based on pathogen-specific effectors
- strong, enduring response

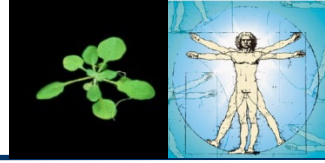
➡ pathogen defense is costly („trade-off“)

Cui, Tsuda, Parker Annual Rev. Plant Biol. (2015)

Pathogen defense in plants and animals:

Similar or different?

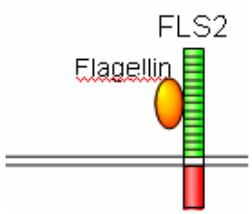
Immunity in plants and animals

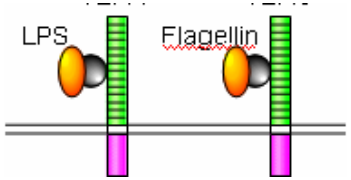


Plants **Animals**

….based on a limited number of genetically encoded PPR

"innate immunity"
PAMP-triggered Immunity:





Resistence

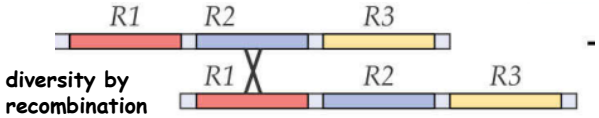
Inflammation…

….based on gene variants:

"aquired" resistance
Effector-triggered Immunity:

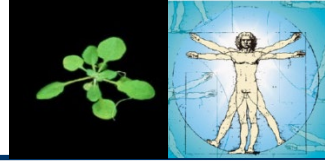
NLR-proteins

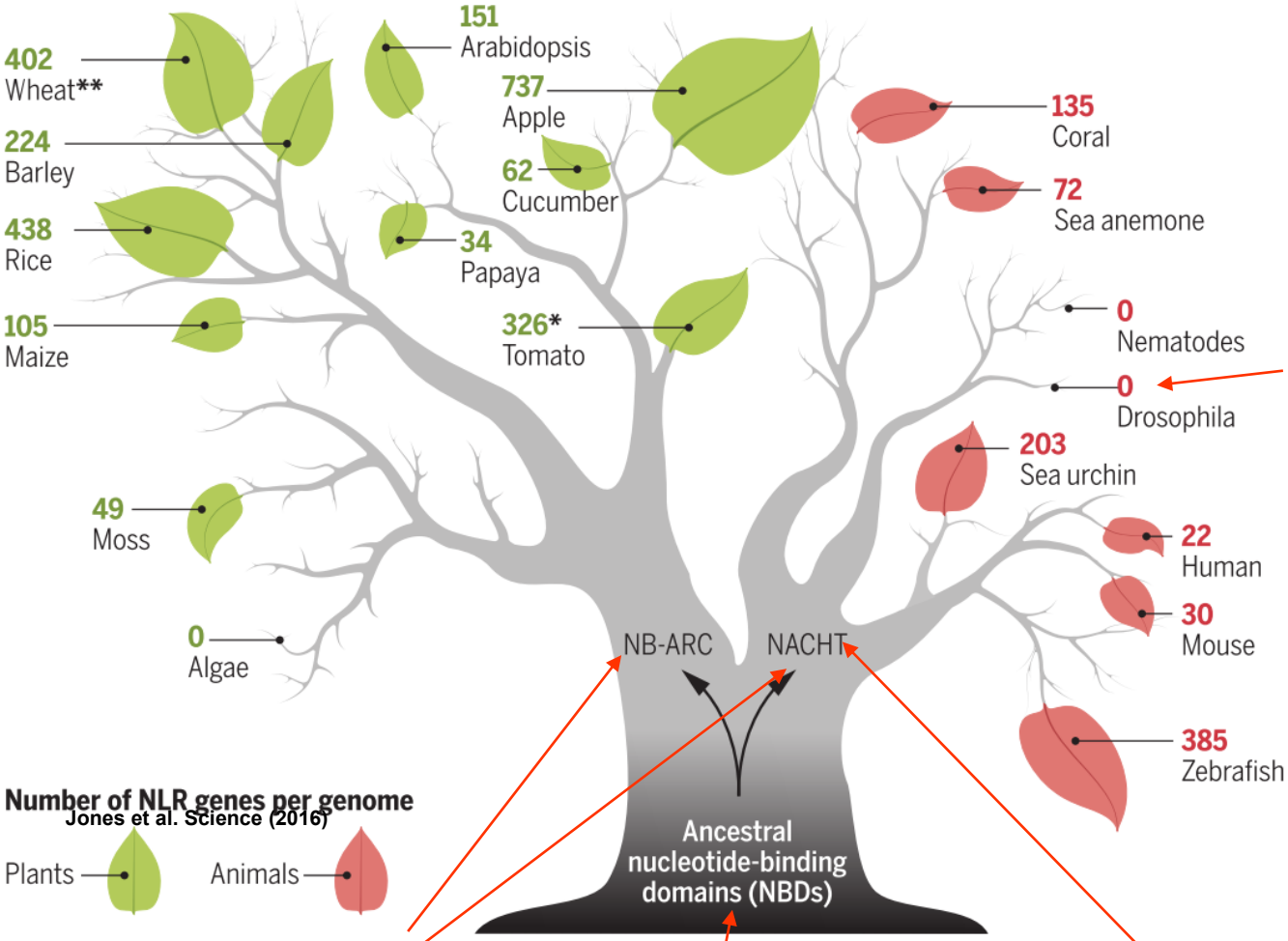
NLR-proteins



NLR-genes occour in plants and animals

(probably not monophyletic)





..novel functions
(e.g. reprocusiton)

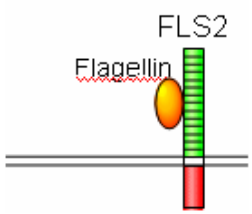
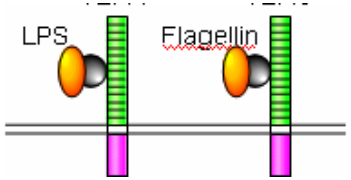
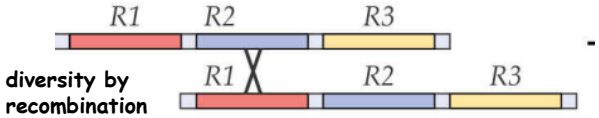
some lineages
have lost NLR

very different in
Domin structure

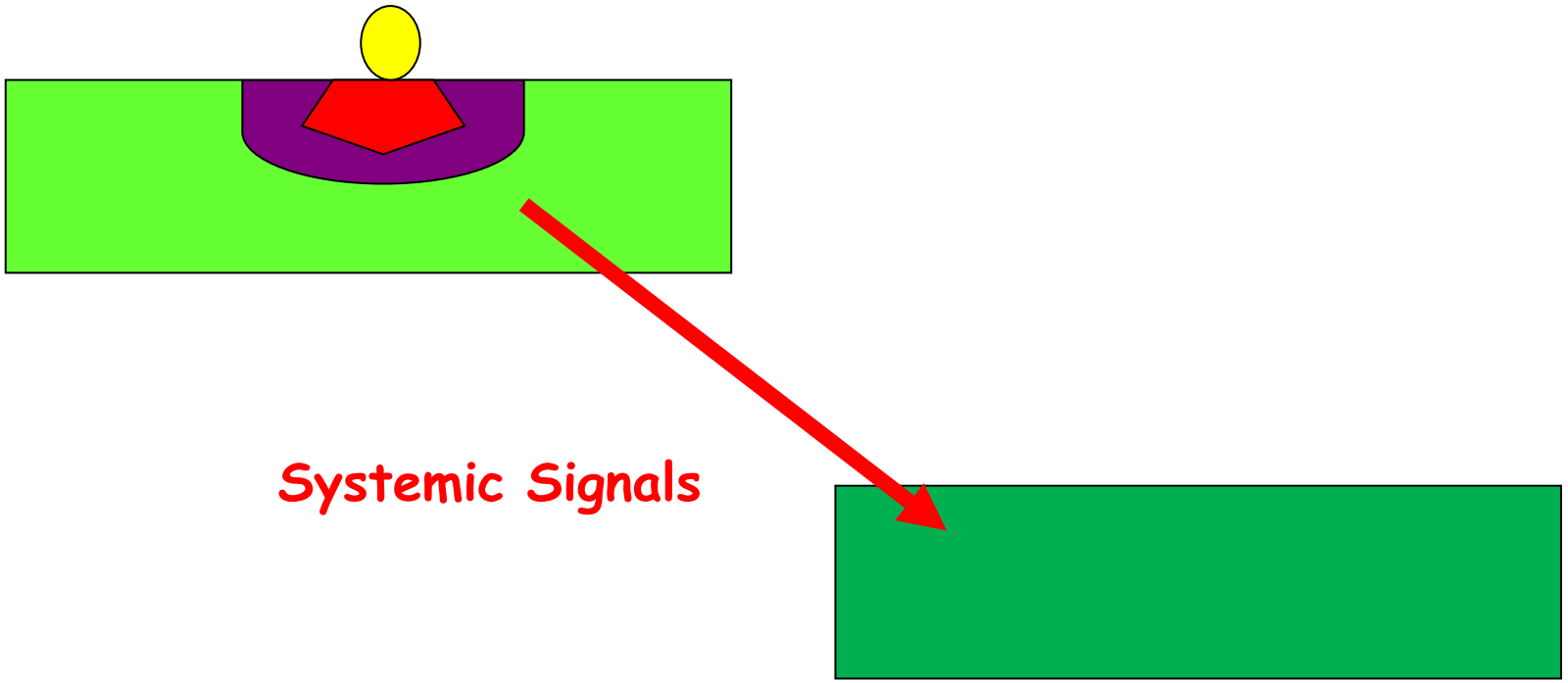
NBD in bacteria

occour very early

Immunity in plants and animals

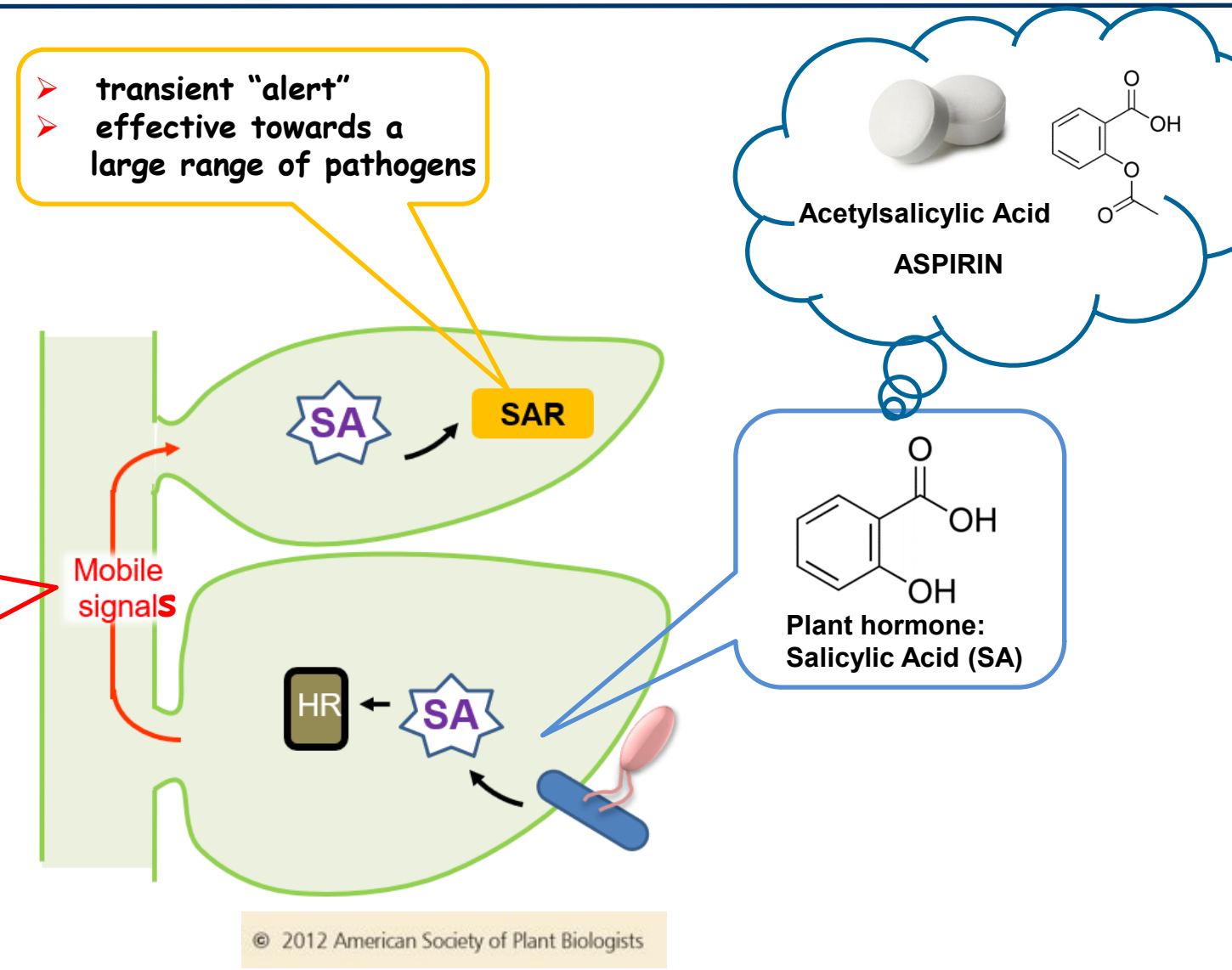
	Plants	*Animals*
	….based on a limited number of genetically encoded PPR	
"innate immunity" PAMP-triggered Immunity:		
	Resistence	Inflammation…
"aquired" resistance Effektor-triggered Immunity:	….based on gene variants:	
	NLR-proteins	NLR-proteins
		
	-	antibody diversity mobile T/B-cells
	systemic aquired Resistance (SAR)	memory

How is Systemic Aquired Resistance (SAR) established?



Systemic Aquired Resistance (SAR)

- transient "alert"
- effective towards a large range of pathogens



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for Plant Biology
University of Würburg

Plant Energy Management – Transcriptional Control – Signaling in Plant Pathogen Interactions