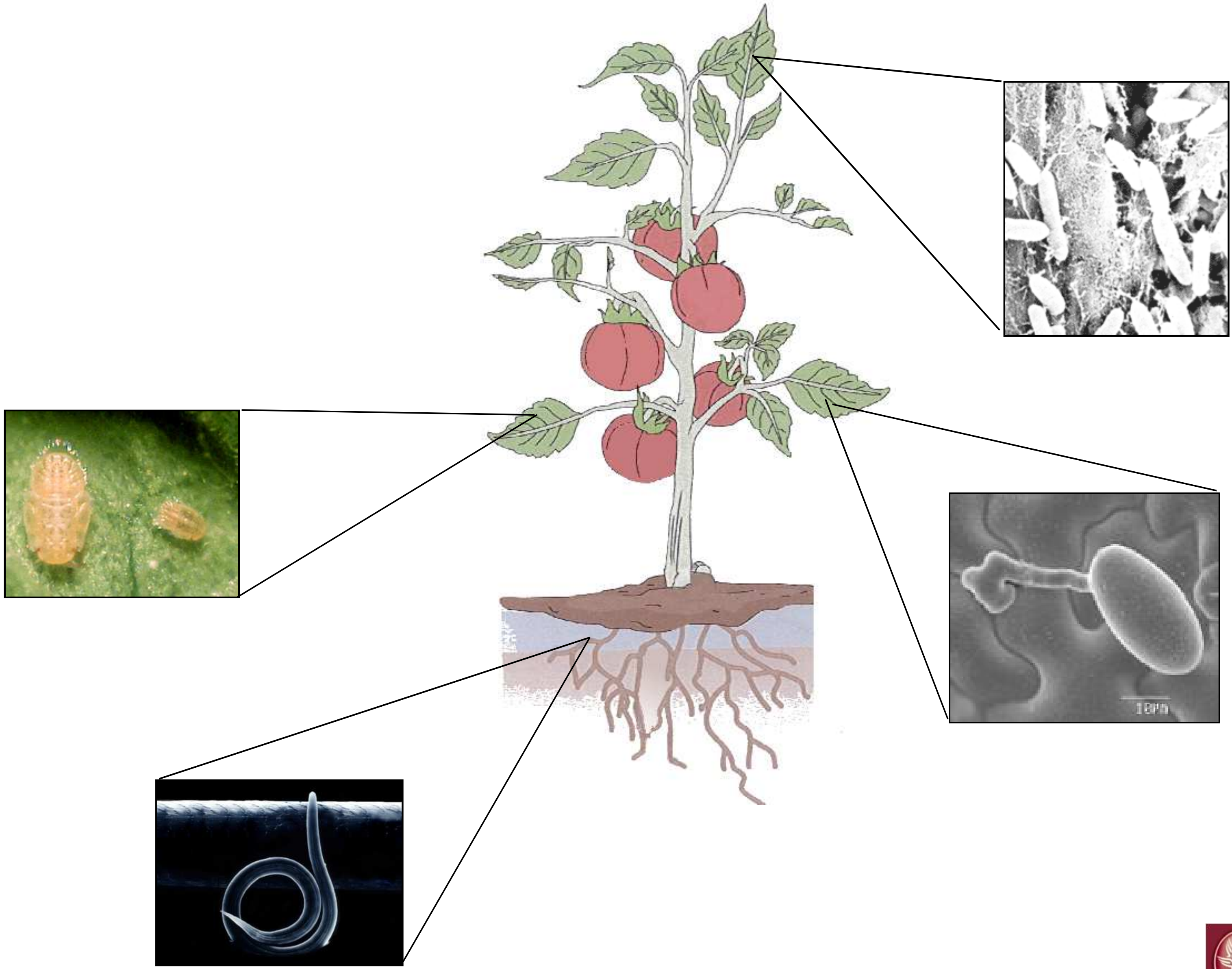
Characterization of LysM-containing receptor-like kinases involved in plant innate immunity

Simone Ferrari

Department of Biology and Biotechnology "Charles Darwin"

Plant-pathogen interactions



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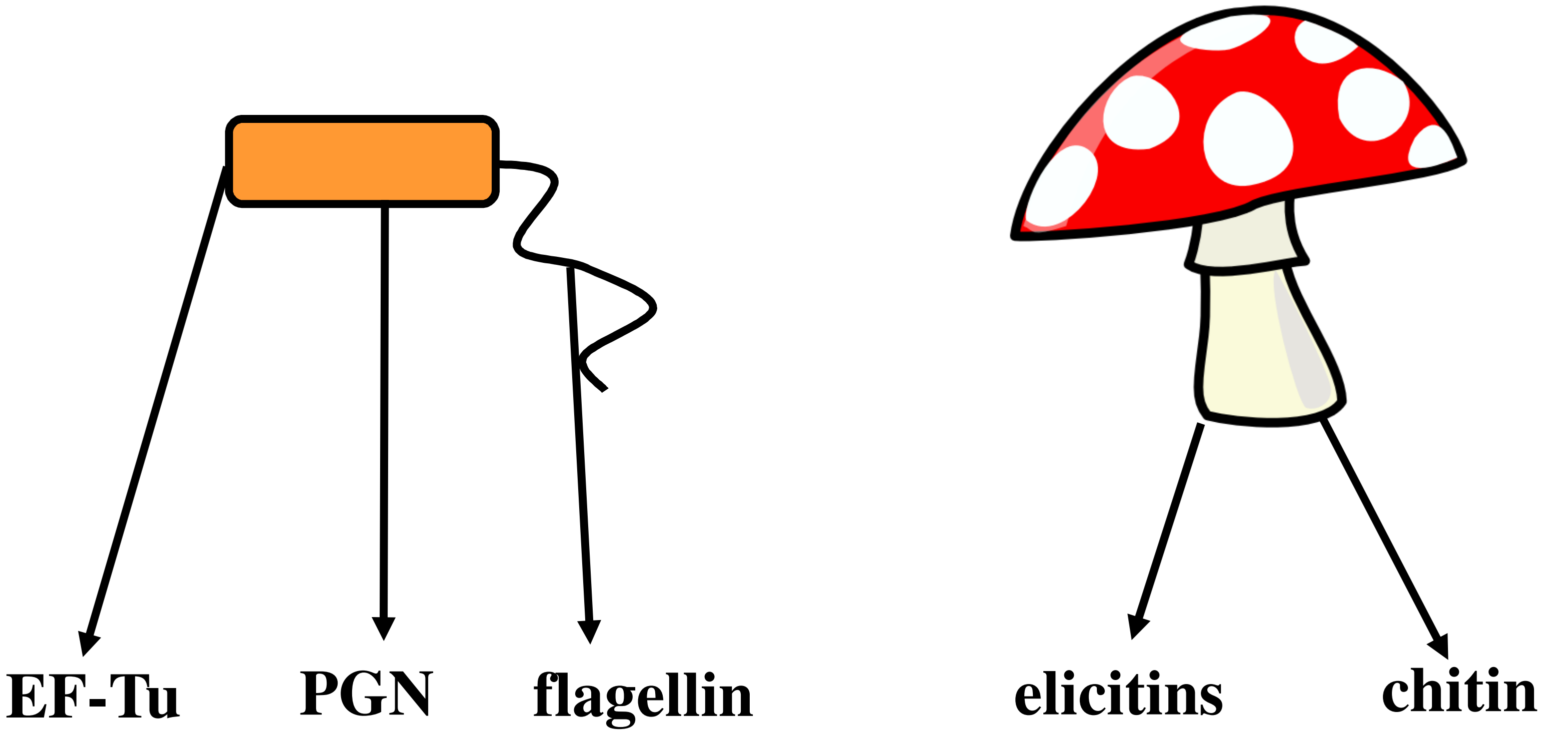
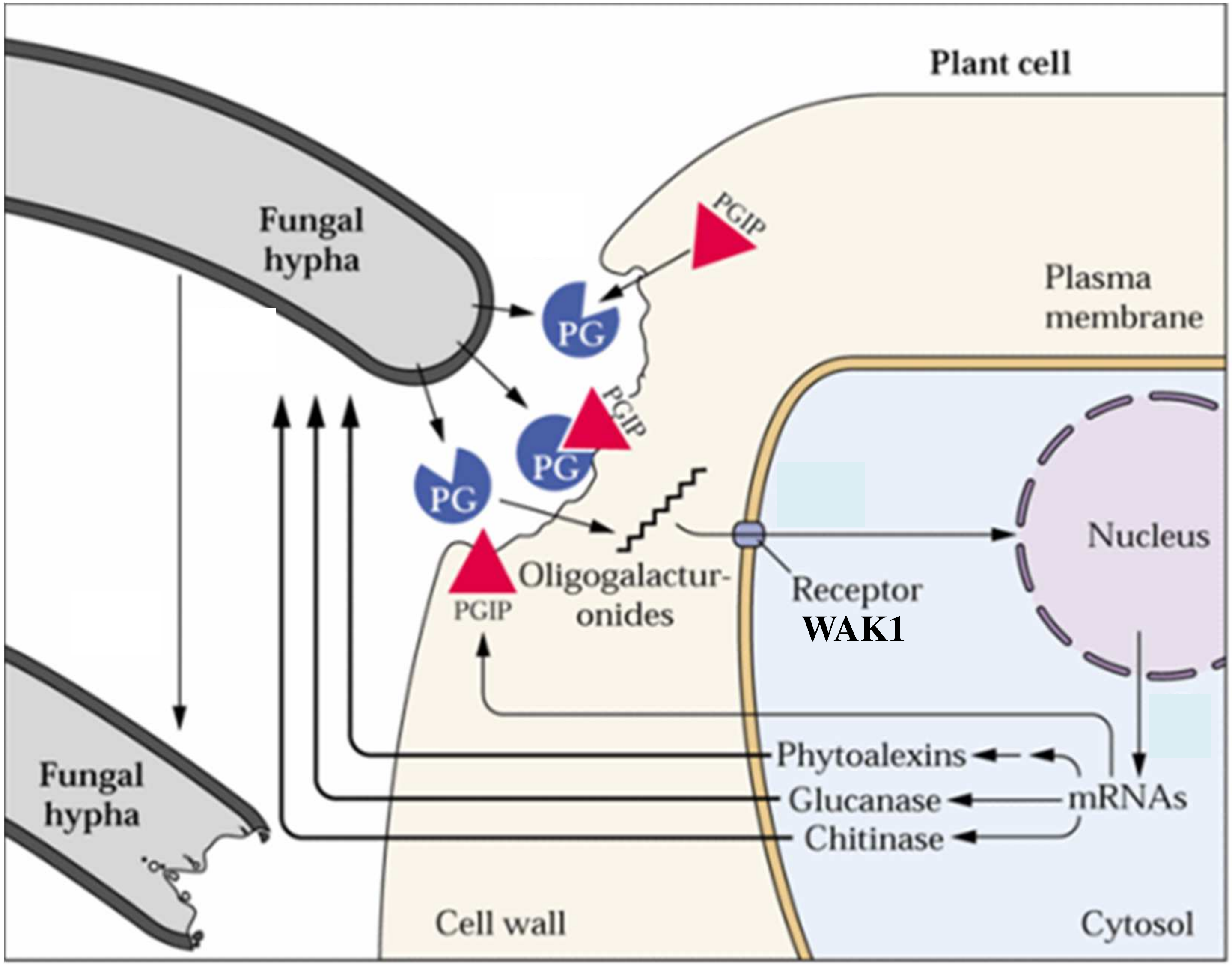
Plants are able to recognize microbe-associated molecular patterns (MAMPs)

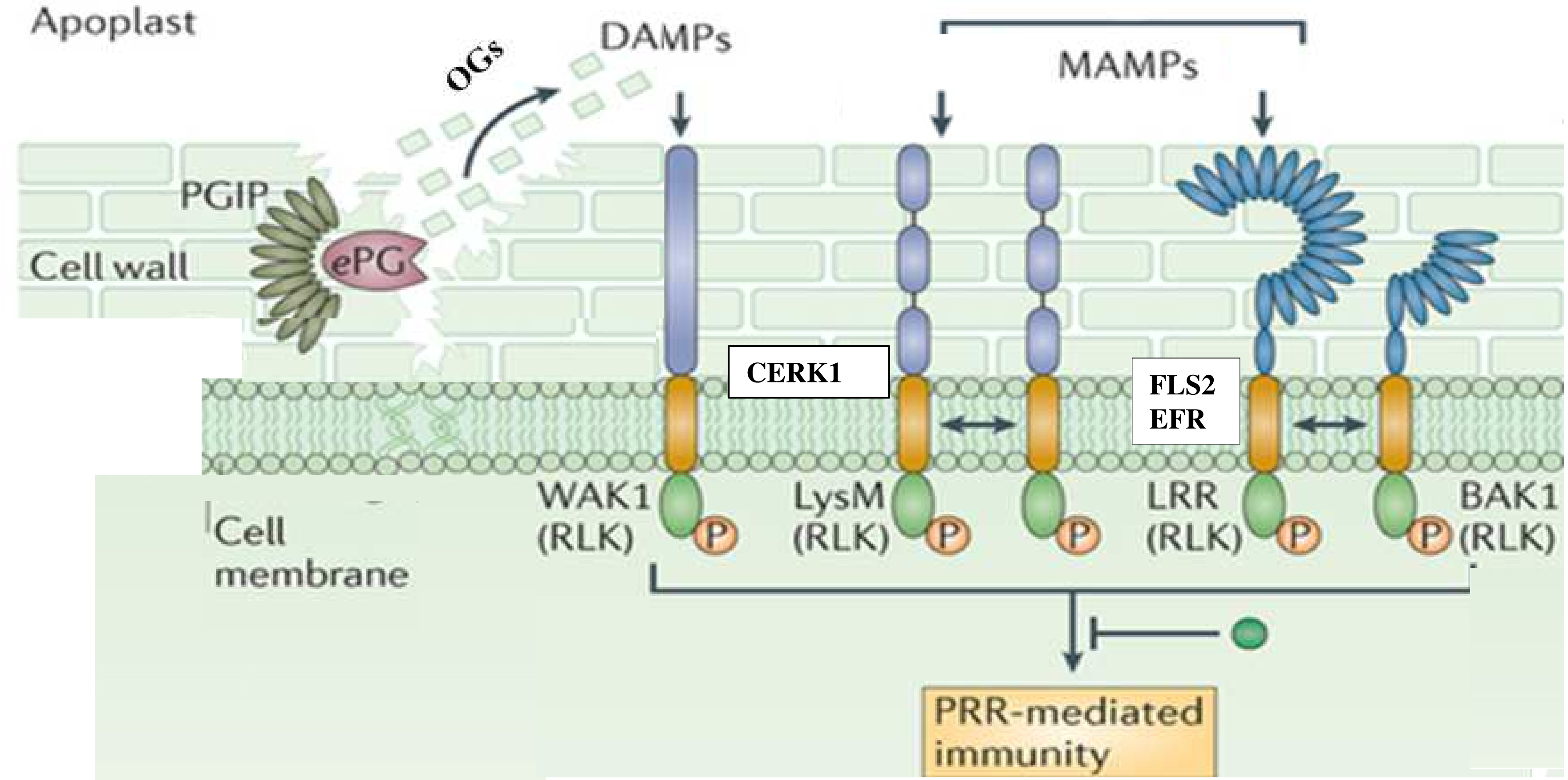


Oligogalacturonides (OGs) are damage-associated molecular patterns (DAMPs)



Adapted by Buchanan et al. (2000)

PRRs: pattern-recognition receptors



LRR-RLKs: receptor like kinases with extracellular Leucine-Rich Repeats (LRRs)

FLS2: receptor of flagellin and its epitope flg22

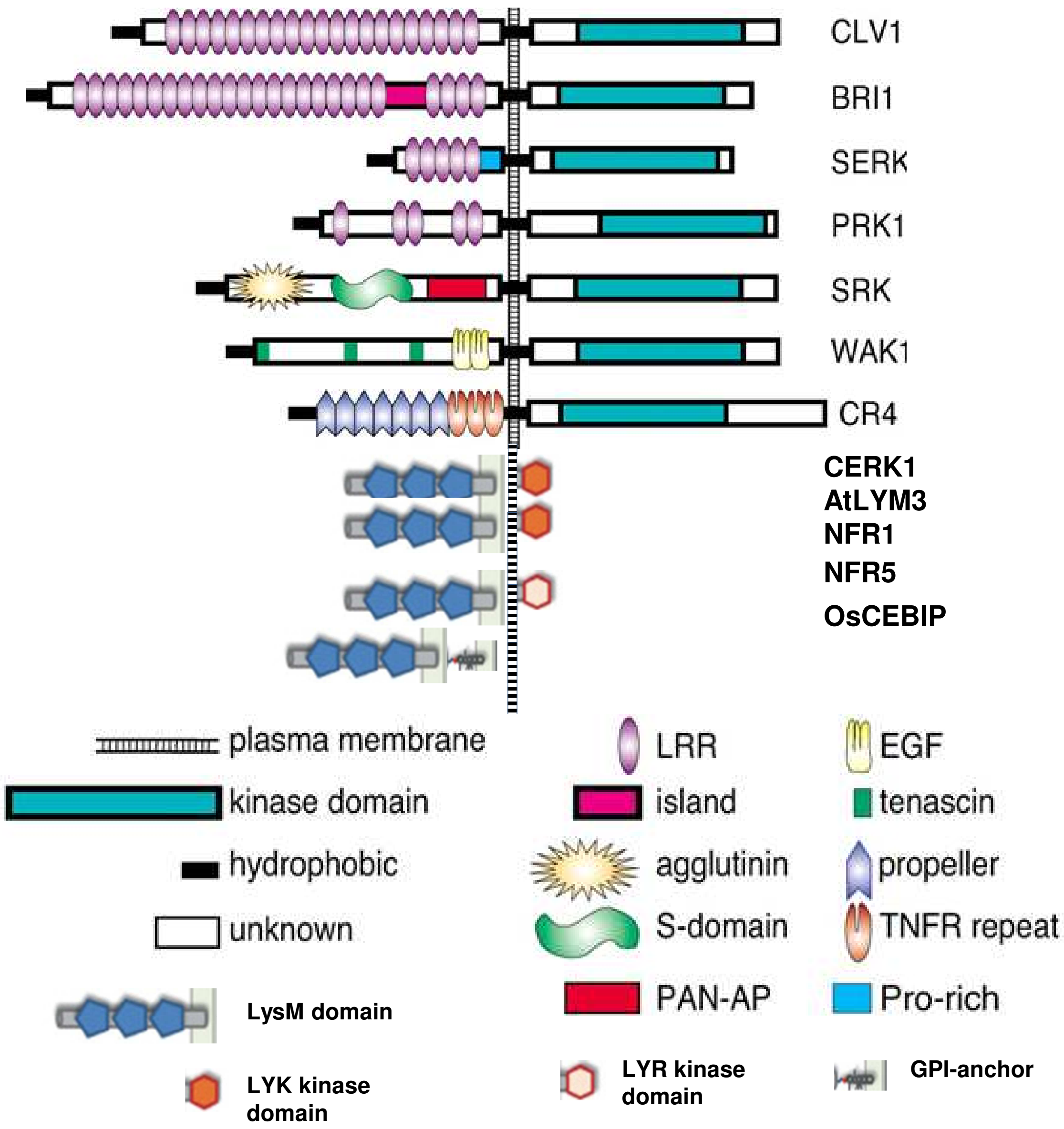
EFR: receptor of elongation factor Tu (Ef-Tu) and its epitope elf18

LysM-RLKs: receptor like kinases with extracellular lysin motifs (LysMs)

CERK1: receptor of chitin fragments

(Adapted by Banfield J.M. et al, Nature Reviews Microbiology 2013)

The Arabidopsis genome encodes a large number of RLKs



- **600 proteins with predicted topologies of RLKs**
- **21 different classes**

↓

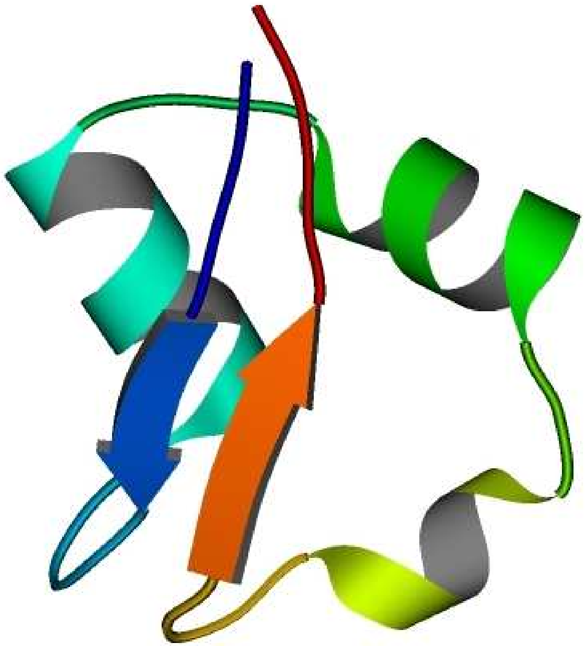
Many of these genes coding for PRRs or their co-receptors

Adapted from Torii K. U. et, al 2000

Project aim

Characterization of LysM containing receptor-like kinases involved in the innate immune response

The lysin motif (LysM)

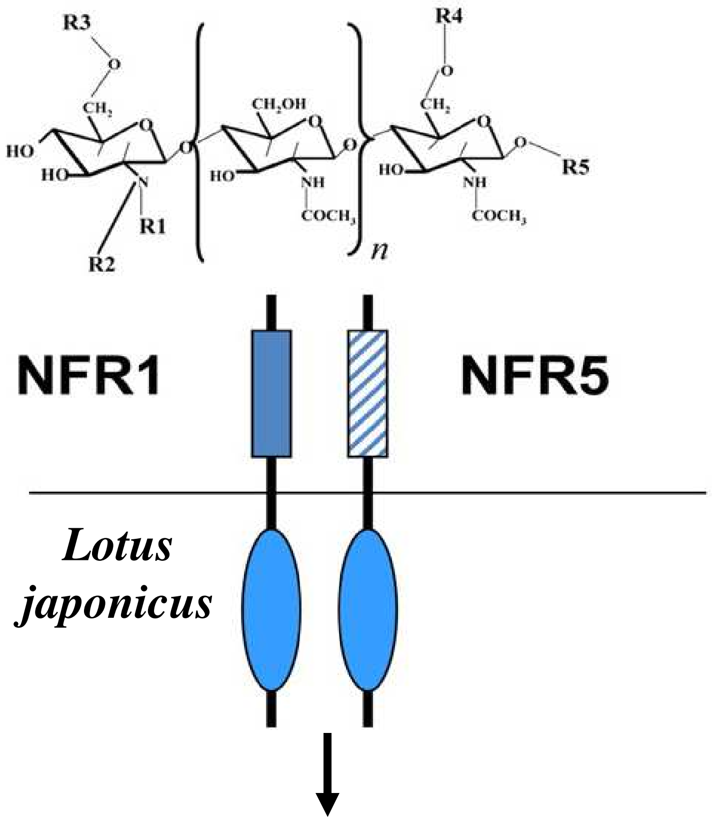


LysM domain of the *E. coli* membrane-bound lytic murein transglycosylase (Bateman and Bycroft, 2000)

- **42-48 amino acids long, is found in all living organisms except in Archaea**
- **Topology βααβ (two α-helices on one side of an antiparallel β sheet)**
- **In Eukaryotes it was first identified in proteins that degrade peptidoglycan (e.g. lysozyme)**
- **LysM-RLK (LYK) proteins are found only in plants**

LysM Receptor-Like Kinases (LYKs)

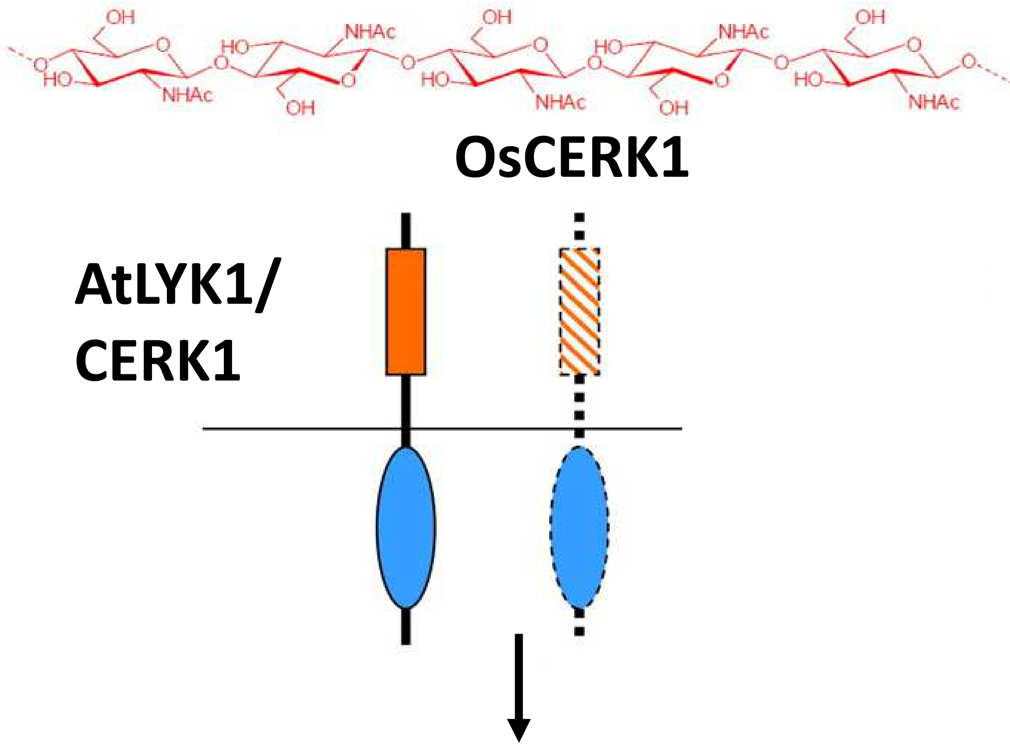
NOD FACTORS



Symbiosis with nitrogen-fixing bacteria

(Radutoiu et al. 2007; Limpens et al. 2003; Madsen et al. 2003)

CHITIN



Innate immunity

(Miya et al. 2007; Shimizu et al. 2010)

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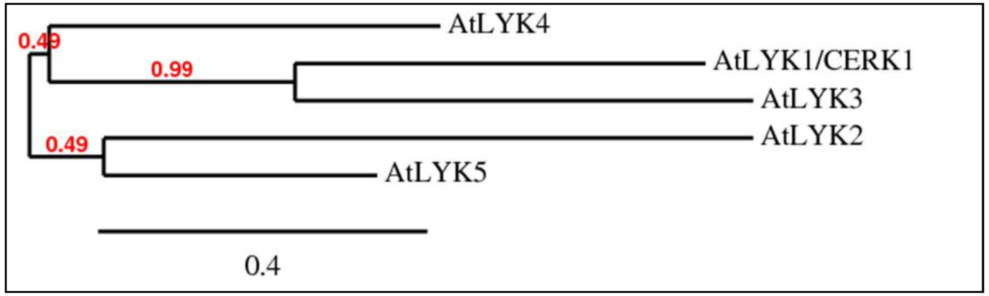
LysM-containing receptors in Arabidopsis

Gene name (other names)	Locus	LysM domain arrangement[a]	Ligand	Receptor Type[f]	Functional kinase? (kinase type)	Mutant phenotype to chitin treatment[i]
AtCERK1	At3g21630	I + II + IV	Chitin[b]	LysM-RLK-I	Yes[g]	Insensitive
(AtLYK1, LysM RLK1)				LYK	(RD kinase)	
AtLYK2	At3g01840	* + * + V	Unknown	LysM-RLK-II	No[h]	Normal
				LYR	(Pseudo kinase)	
AtLYK3	At1g51940	* + VII + *	Unknown	LysM-RLK-I	Yes[h]	Normal
				LYK	(RD kinase)	
AtLYK4	At2g23770	I + II + III	Chitin[c]	LysM-RLK-II	No[g]	Moderately insensitive
				LYR	(Pseudo kinase)	
AtLYK5	At2g33580	I + II + III	Chitin[c]	LysM-RLK-II	No[h]	Normal

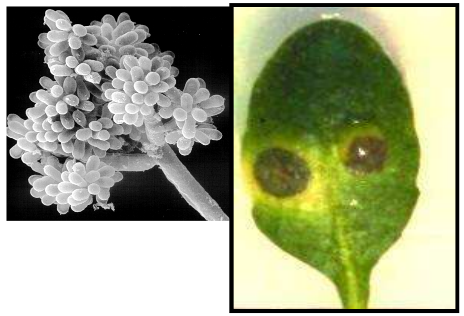
(Adapted from G. Stacey et. al, 2013)

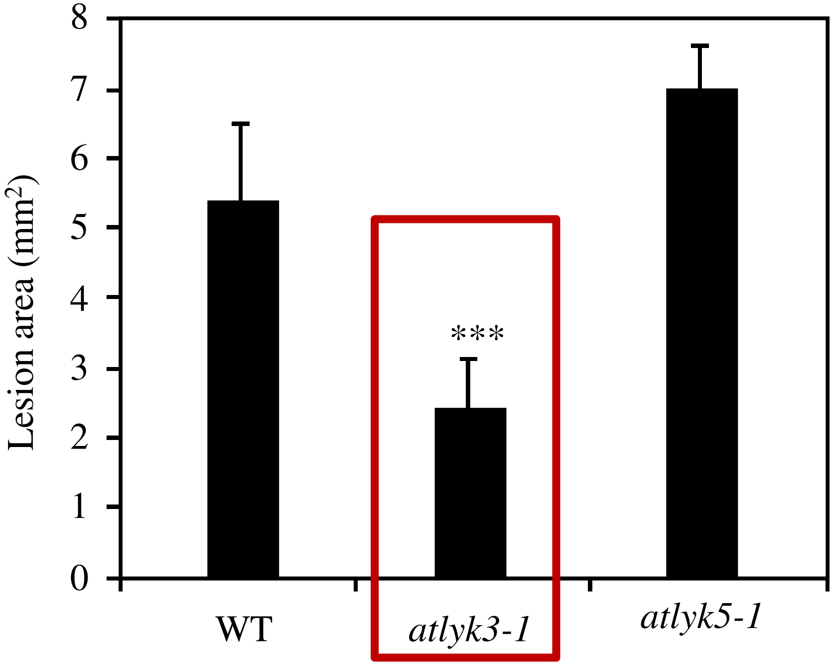


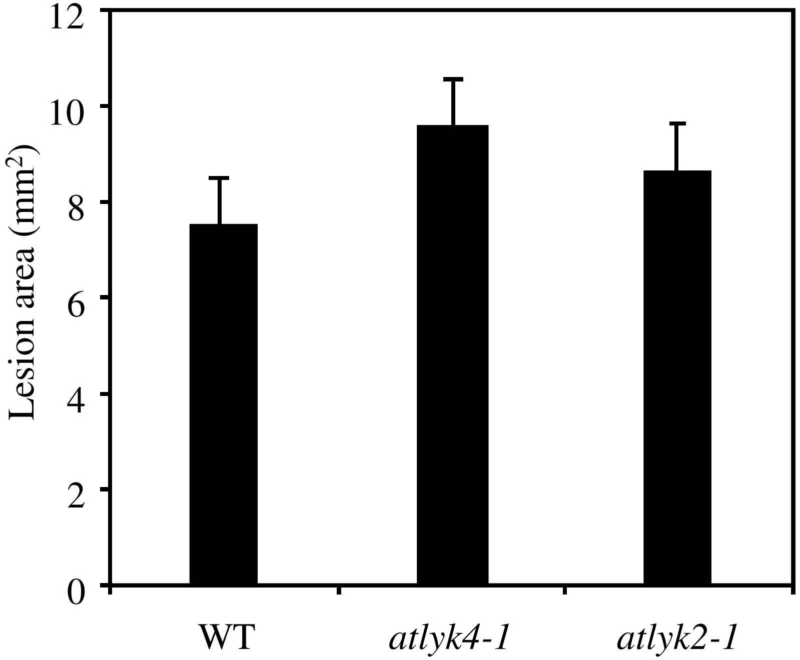
Phylogenetic tree built on the basis of the whole protein sequence alignment (http://www.phylogeny.fr/)

Resistance to *Botrytis cinerea* in *atlyk* insertional mutants

B.cinerea







Rosette leaves were inoculated with *B. cinerea*, and lesion areas were measured after 48 h (*, P ≤ 1×10^{-4})**


Functional characterization of *AtLYK3*
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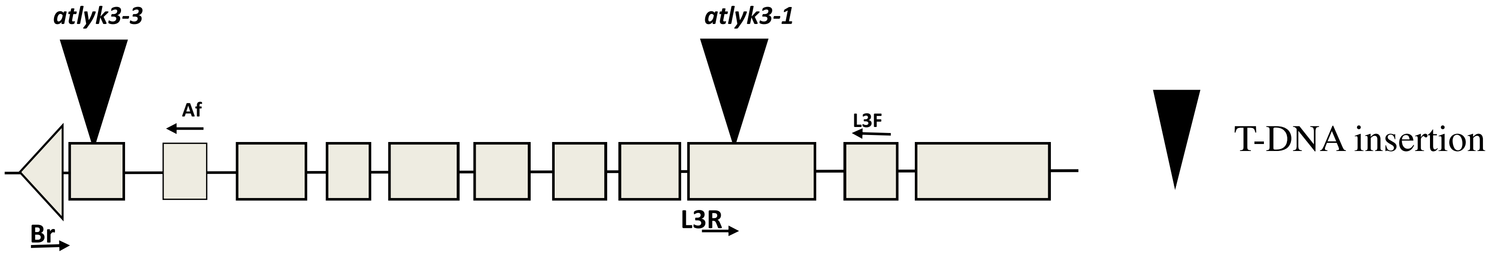
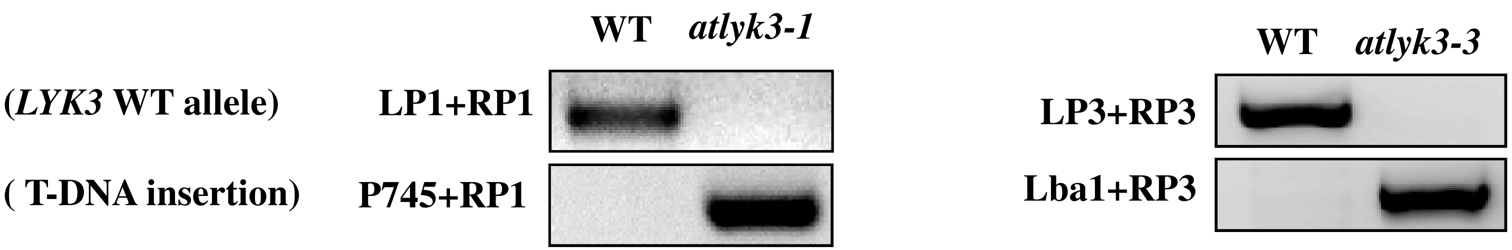
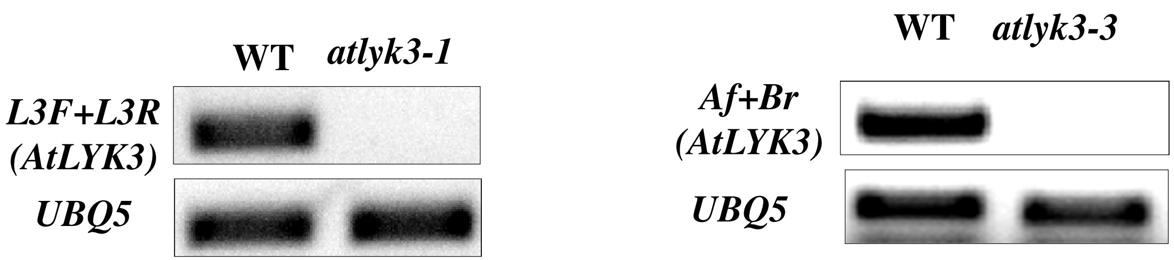
Isolation of insertional mutants for *AtLYK3*



Genotyping:



Level of expression of the transcript :



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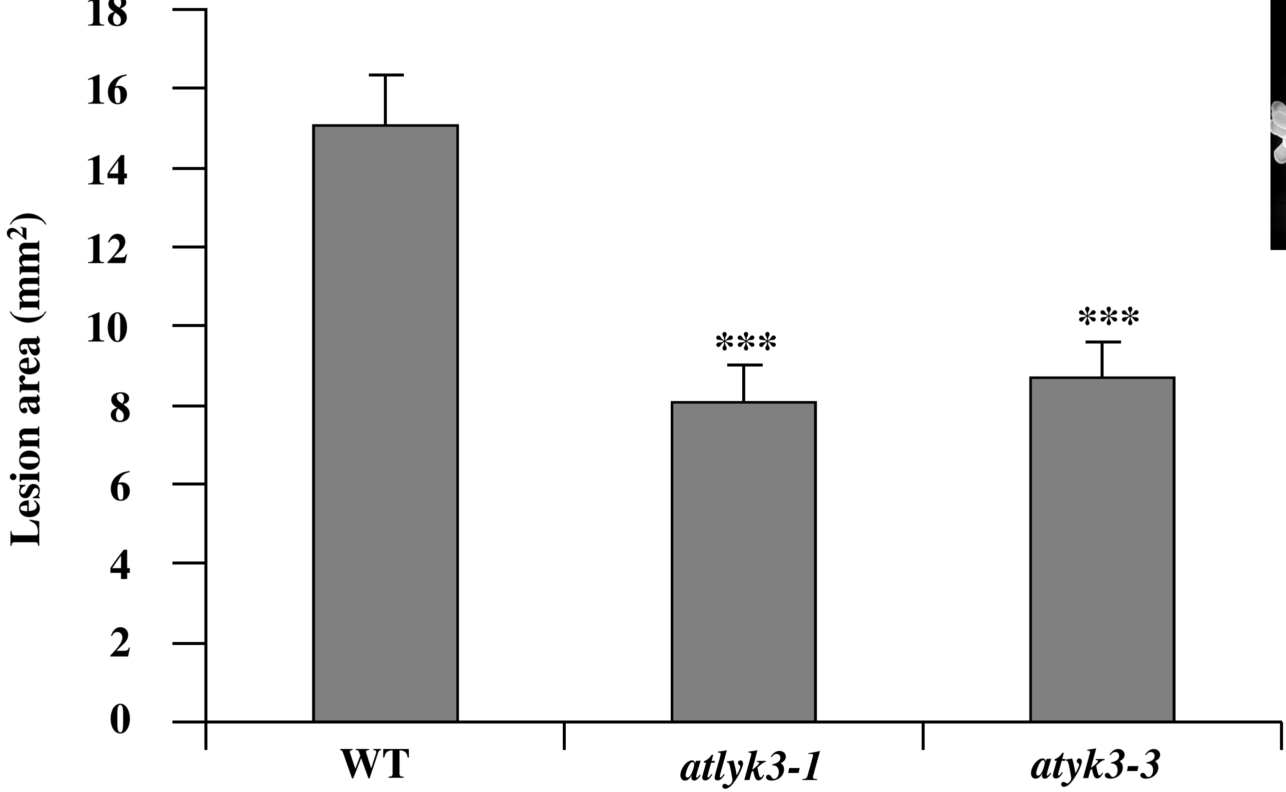
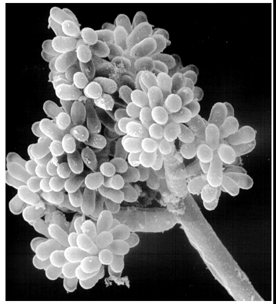
atlyk3 mutants are more resistant to *B. cinerea* infection



B.cinerea



WT, *atlyk3-1* and *atlyk3-3* rosette leaves were inoculated with *B. cinerea* for 48 h, and lesion areas were measured.
(*, $P \leq 1\times10^{-4}$)**

(Paparella et. al, 2014)

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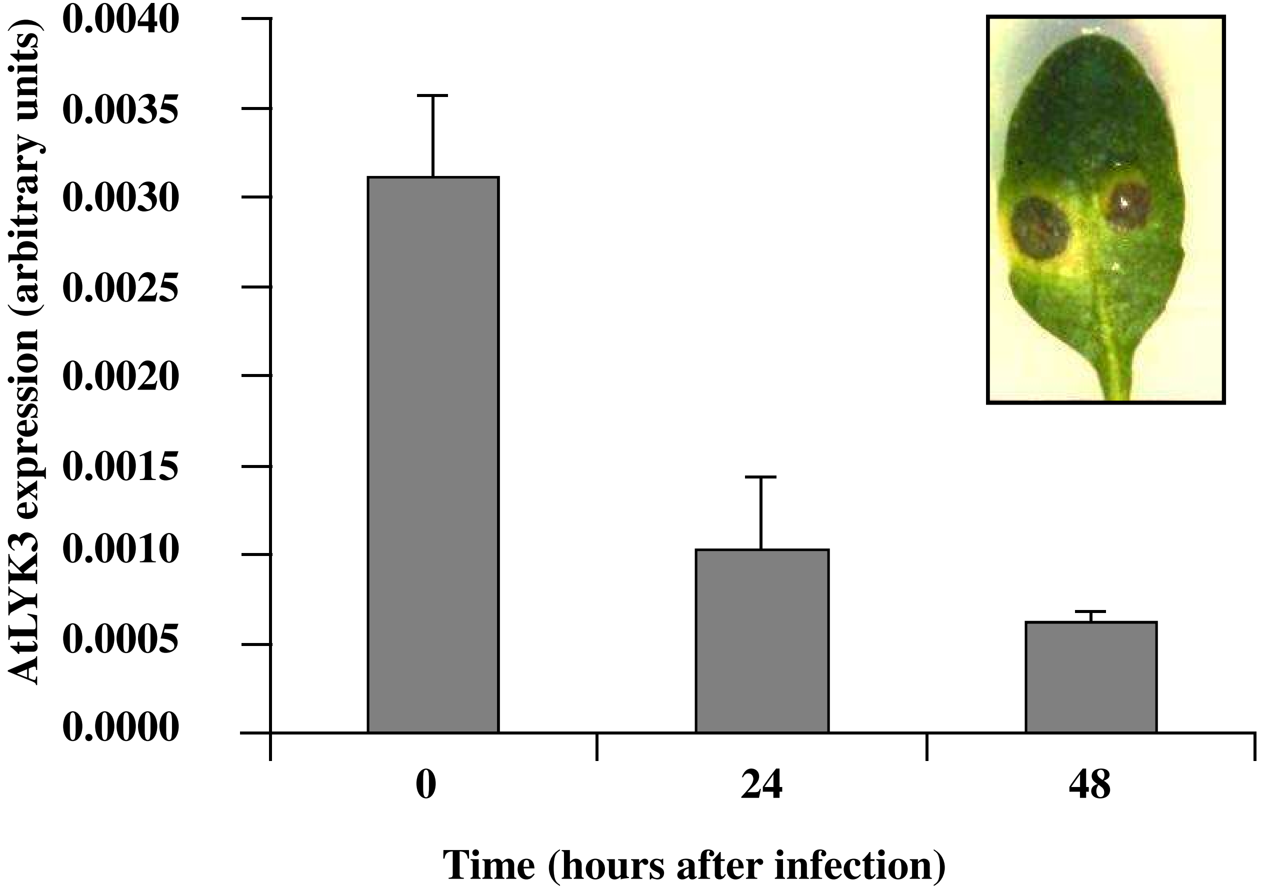
Expression of *AtLYK3* is repressed during *Botrytis cinerea* infection



Leaves were inoculated with *B.cinerea* spores $5x10^{5}$ sp ml^{-1} for 24 and 48 h qRT-PCR with *UBQ5* as a reference gene

(Paparella et. al, 2014)

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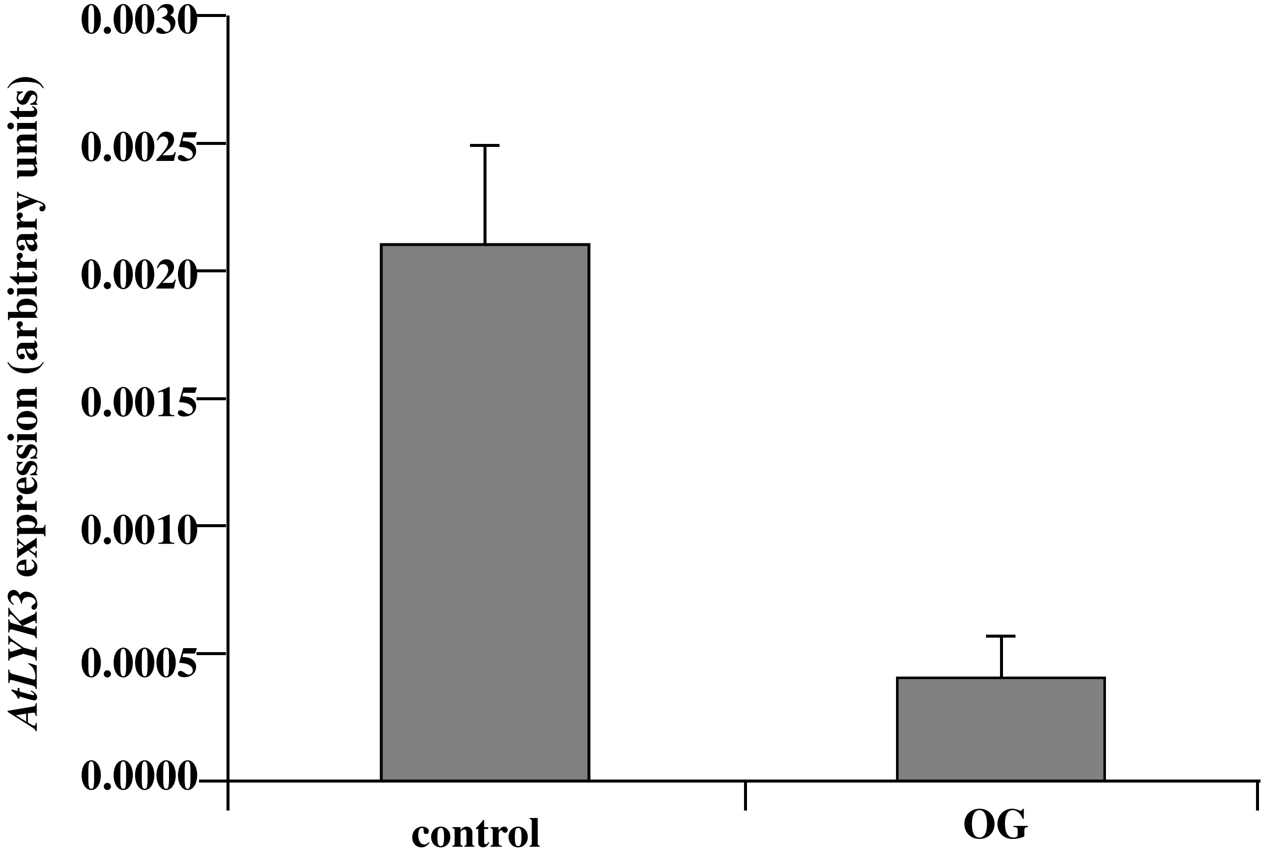
Expression of *AtLYK3* is repressed by DAMPs such as OGs



qPCR in seedlings treated for 3h with water (control) or OG 100 µg ml^{-1}
UBQ5 gene was used as reference.

(Paparella et. al, 2014)

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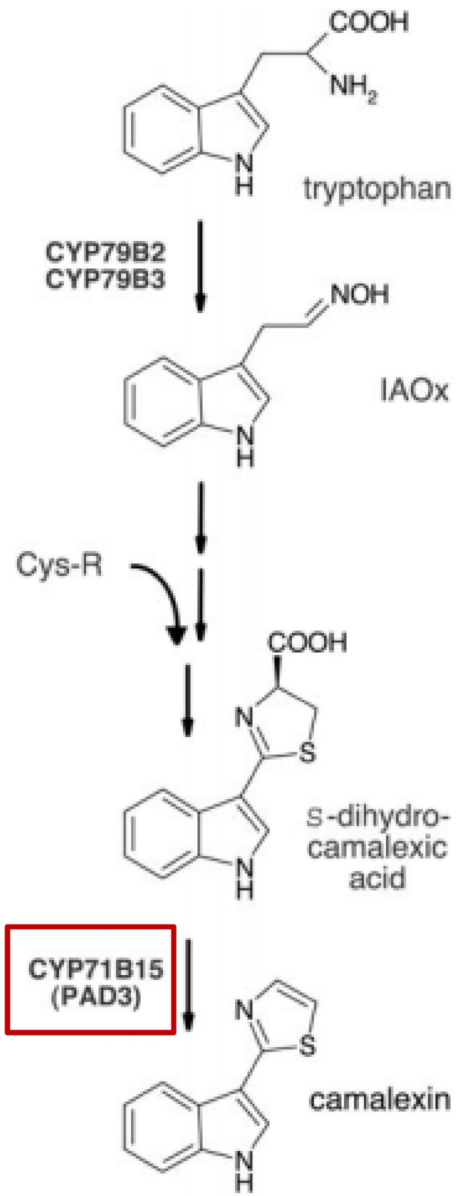
Camalexin contributes to resistance against *B.cinerea* in *Arabidopsis*



(Erich Glawischnig et al 2006)

- **CYP71B15 (*PAD3*) (Zhou et. al, 1999)**
- ***PAD3* is required for resistance against *B. cinerea* (Ferrari et. al, 2003)**
- ***PAD3* expression is strongly up-regulated by elicitors and fungal infection (Ferrari et. al, 2007)**

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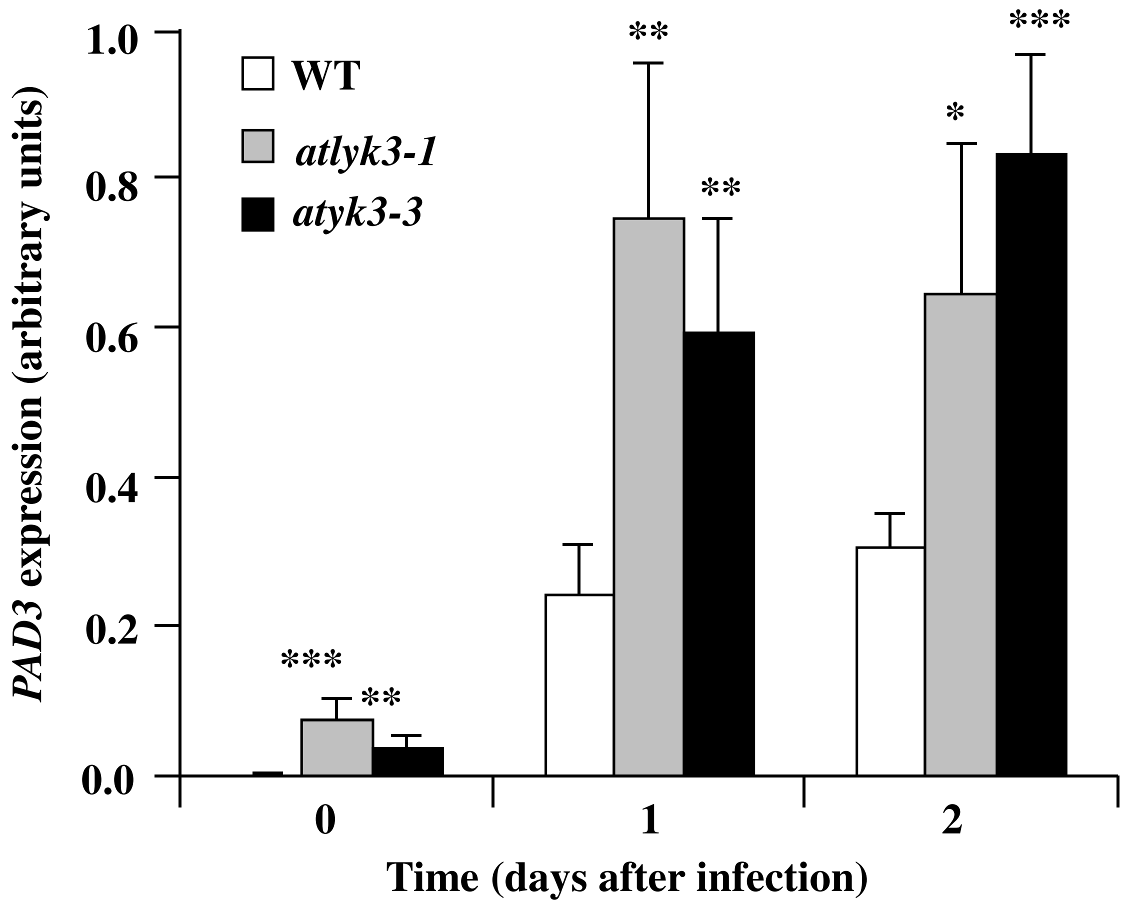
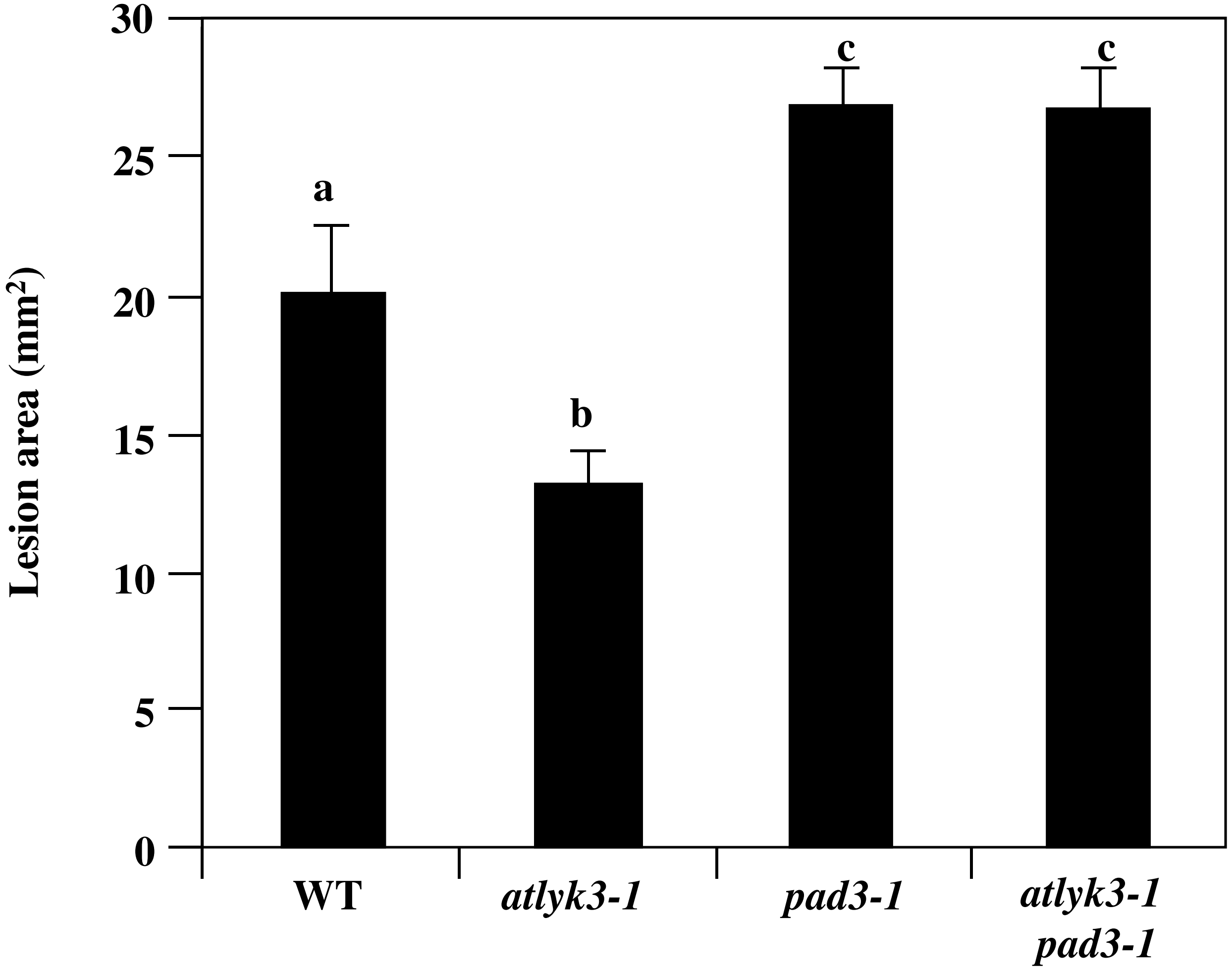
Loss of AtLYK3 de-represses expression of *PAD3*



qPCR (*UBQ5* as reference) of rosette leaves inoculated with *B. cinerea*. Total RNA was extracted at the indicated times. (t-test: *, P<0.05; **, P< 0.02; ***, P< 0.01).

(Paparella et. al, 2014)

Enhanced resistance against *B. cinerea* of *atlyk3-1* plants is dependent on *PAD3*



WT, *atlyk3-1*, *pad3-1* and *atlyk3-1 pad3-1* rosette leaves were inoculated with *B. cinerea* and lesion areas were measured 48 h after inoculation (n ≥ 10); Tukey's HSD test (P ≤ 0.01).

(Paparella et. al, 2014)

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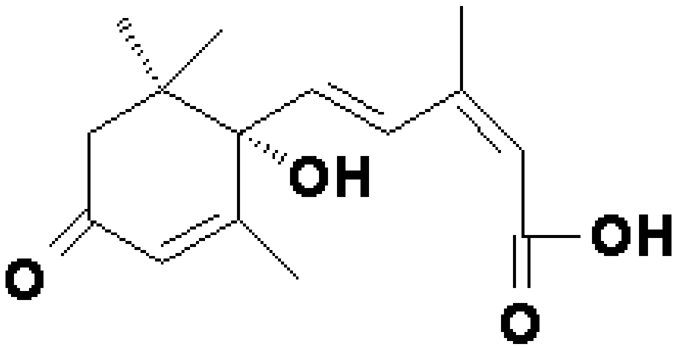
Abscisic acid (ABA) is a negative regulator of resistance against *B. cinerea*

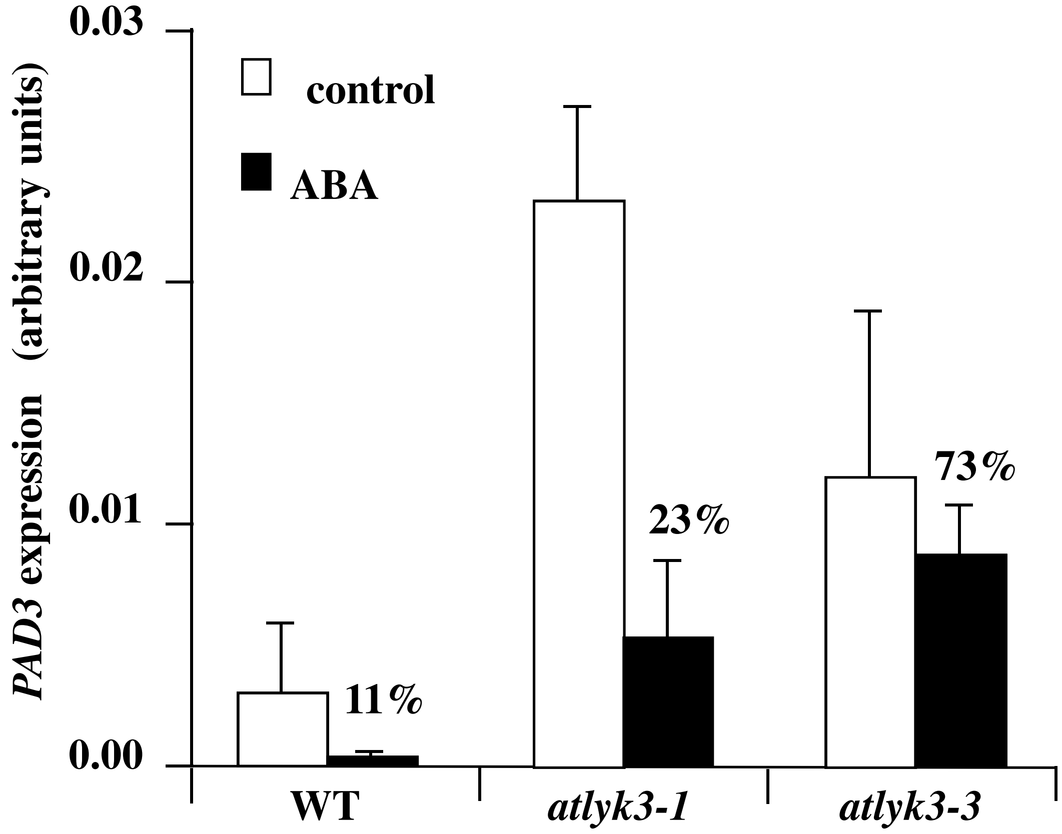


• ABA is important for plant physiology and resistance to abiotic stresses

• Mutants impaired in the biosynthesis or in the transduction of this hormone display increased resistance to *B. cinerea (*Adie et al. 2007)

• ABA pretreatments repress elicitor-induced expression of several defense related gene (Clay et al. 2009)

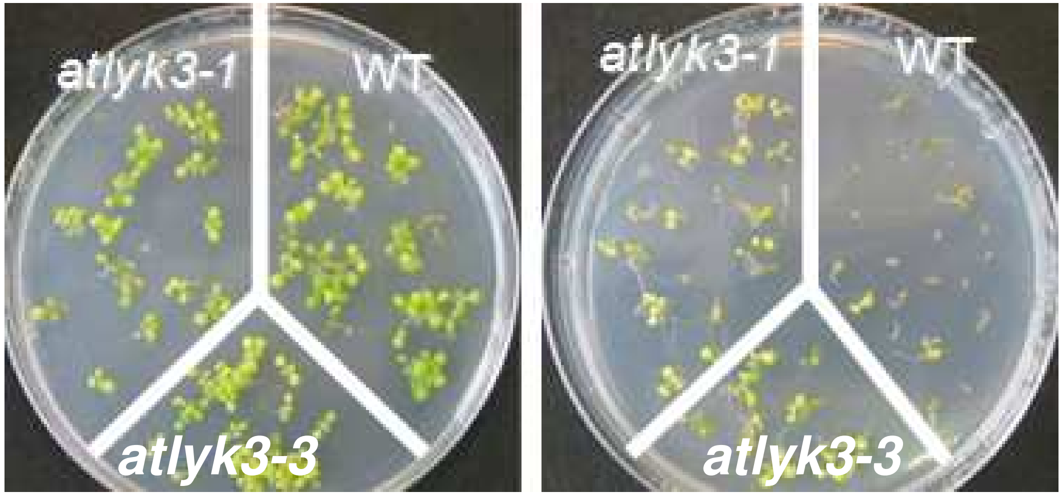
AtLYK3 is required for ABA-mediated repression of *PAD3*



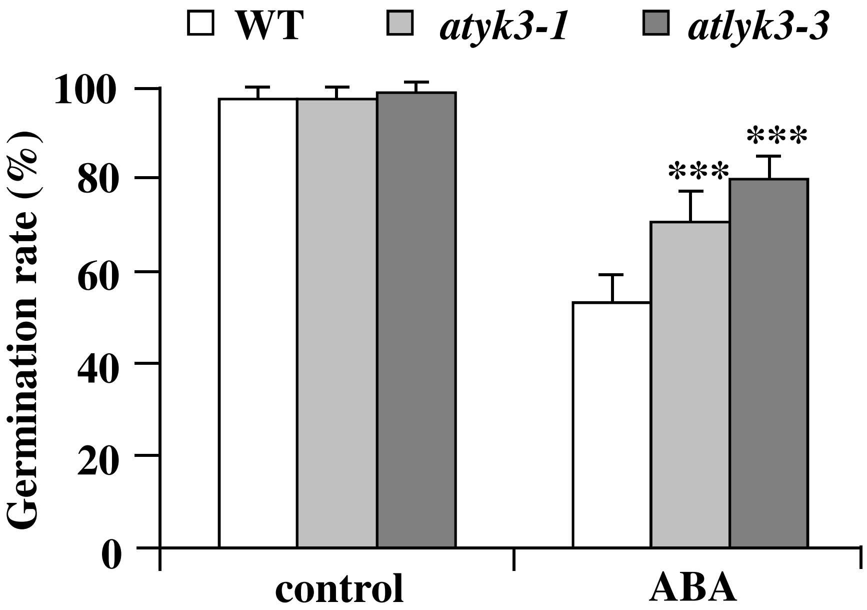
Seedlings were treated for 24 h with 0.01% MeOH (control) or 10 µM ABA (black bars)
qPCR, using *UBQ5* as reference

(Paparella et. al, 2014)

AtLYK3 is required for ABA-induced inhibition of germination



control ABA



Germination rate was determined three days after sowing on plates containing 0.05% MeOH (control) or 5 µM ABA.

(n>30 for each experiment)

(Paparella et. al, 2014)

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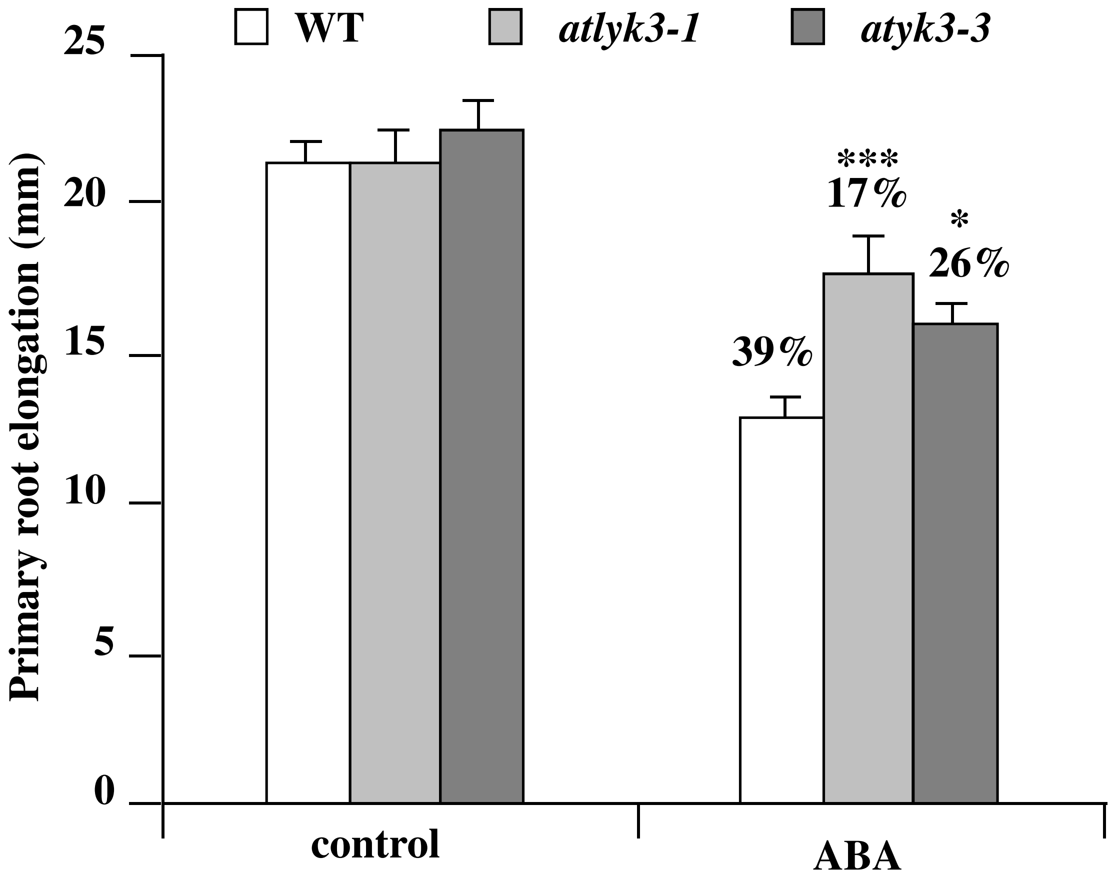
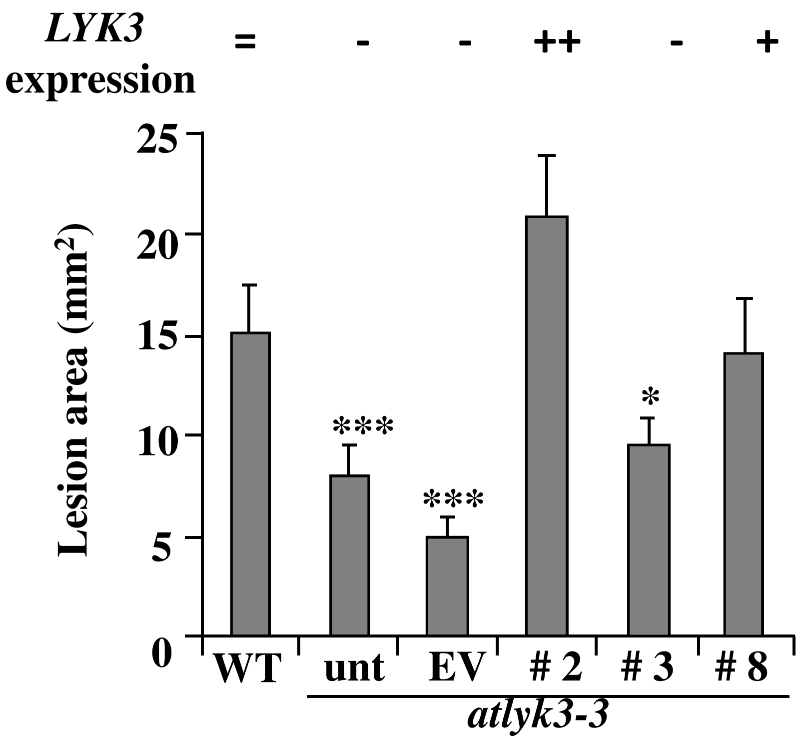
AtLYK3 is required for ABA-induced inhibition of root elongation

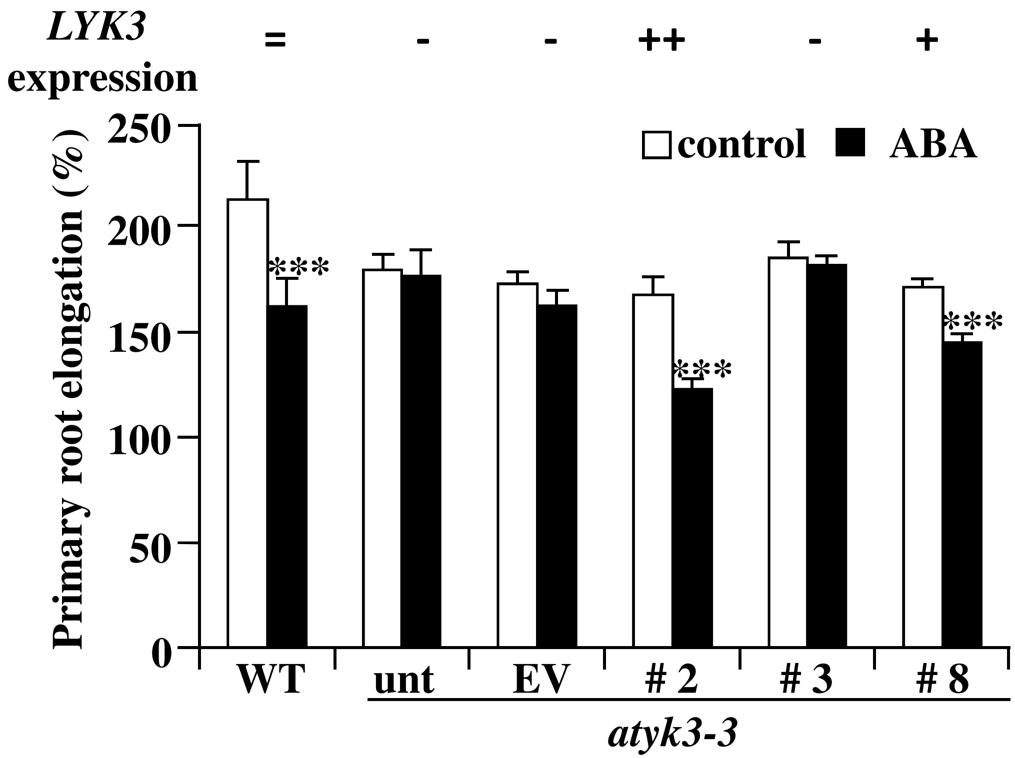


0.05% MeOH (control) or 2.5 µM ABA.
Elongation of the primary root was measured after two days. (n> 10) Student's t-test (*, P < 0.01).**

(Paparella et. al, 2014)

AtLYK3-GFP complements *atlyk3-3* phenotypes





WT, unt, EV, #2,#3,#8 rosette leaves were inoculated with *B. cinerea* for 48 h and lesion area was determined 72 h after infection

0.05% MeOH (white bars) or 5 µM ABA (black bars). Asterisks indicate significant differences between control- and ABA-grown seedlings, according to Student's t-test (*, $P < 0.01$)**

EV: empty vector
Unt: *atlyk3-3* untransformed

#2: high expression (3:1)
#3: no expression (15:1)
#8: low expression (3:1)

(Paparella et. al, 2014)

Does ABA regulates *AtLYK3* expression?

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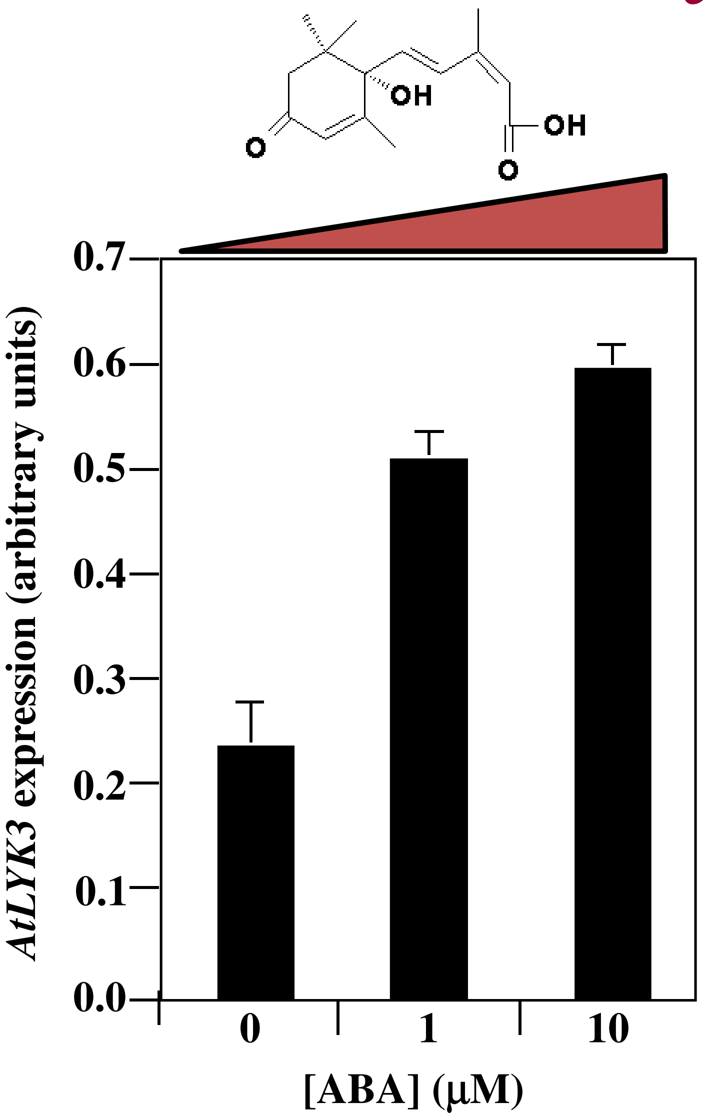
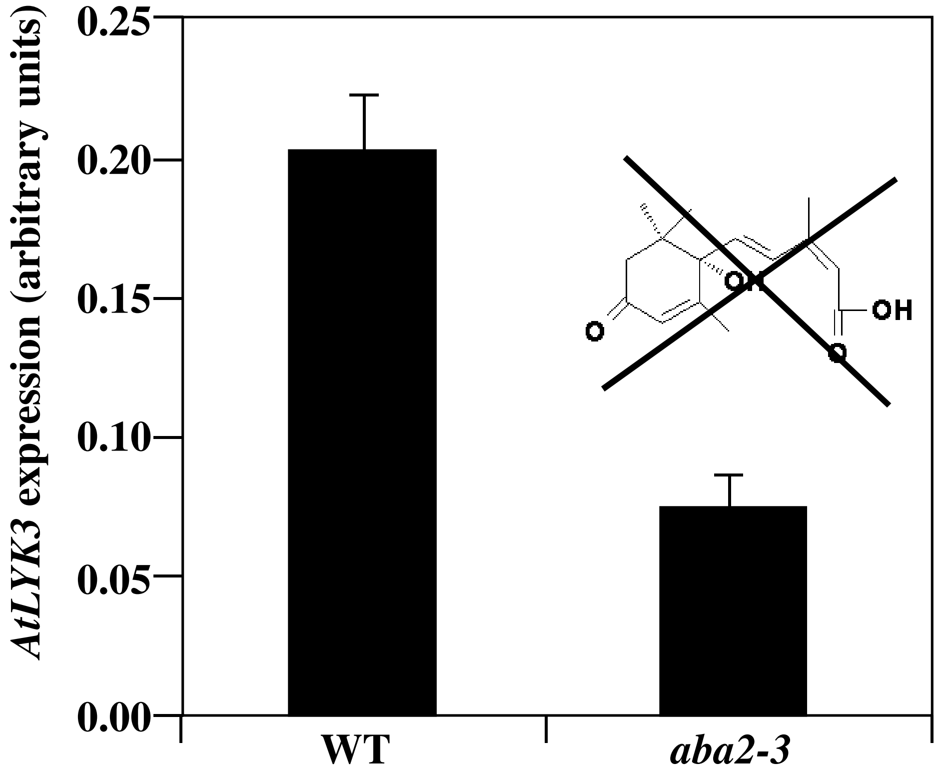
Expression of *AtLYK3* is positively regulated by abscisic acid (ABA)





(Paparella et. al, 2014)

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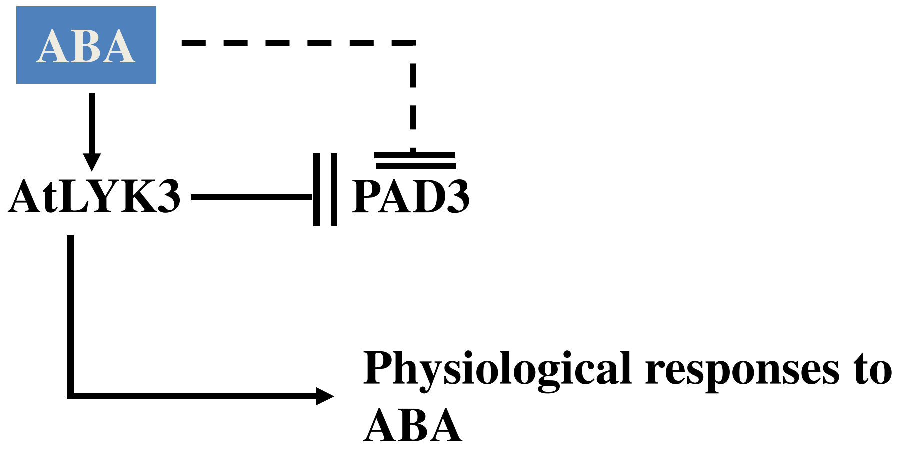
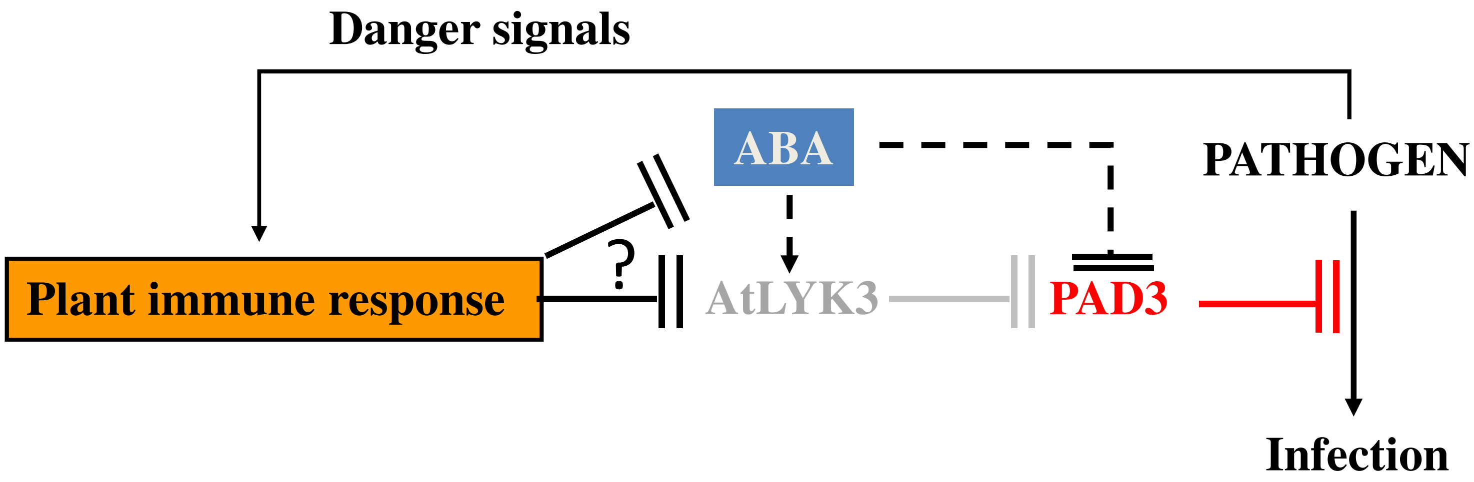
A model for the role of AtLYK3

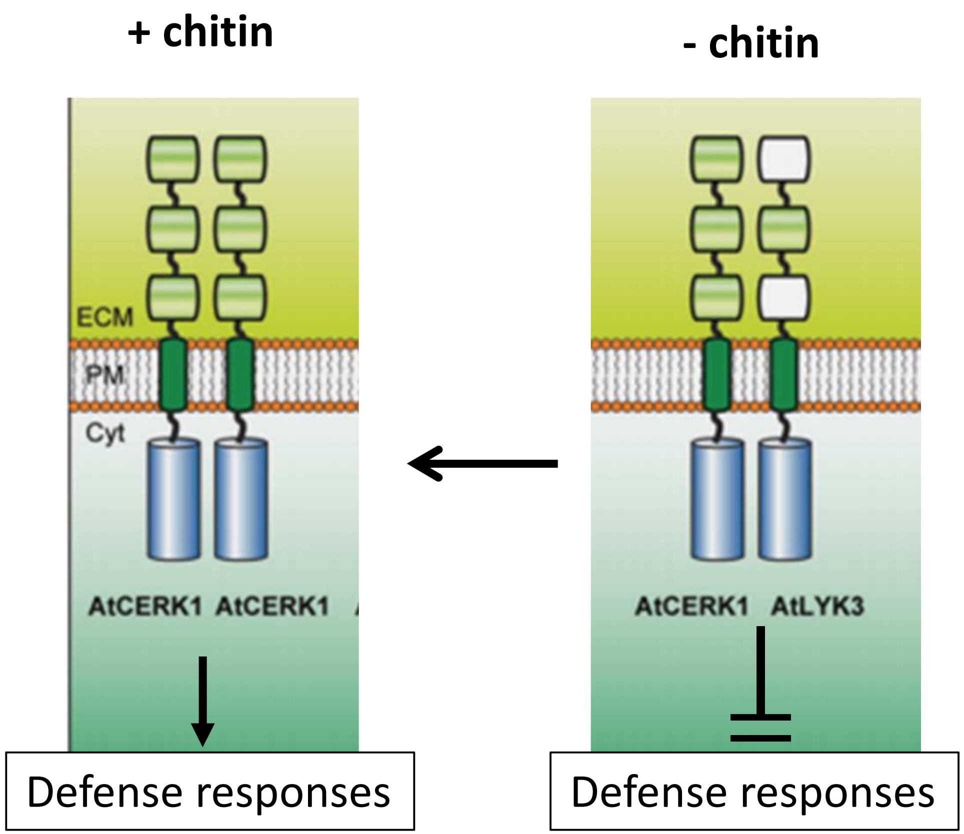
No pathogen:



A model for the role of AtLYK3

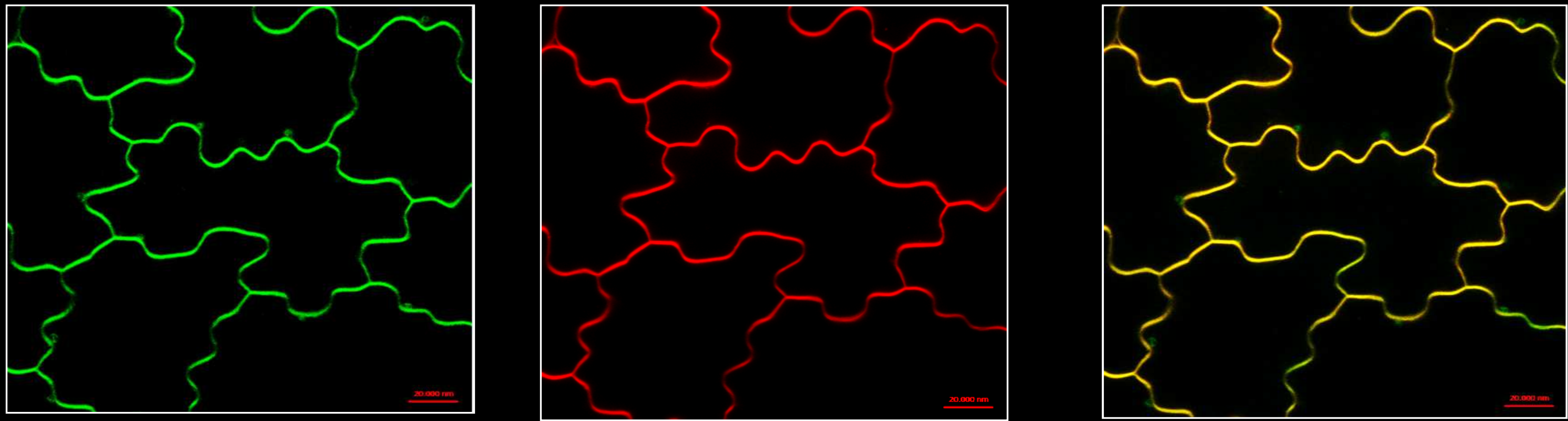


AtLYK3 may interact to AtCERK1



(adapted from G. Stacey et al 2013)

AtLYK3-GFP localizes in the plasma membrane



GFP FM4-64 merge

(Paparella et. al, 2014)

Summary (I)

-AtLYK3 negatively regulates the expression of defense genes and resistance to pathogens

-The expression of *AtLYK3* is repressed by elicitors and infection, while it is induced by ABA

- Plants lacking a functional AtLYK3 show alterations of some ABA-dependent responses, including repression of *PAD3* expression

- AtLYK3 localizes at the plasma membrane

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Conclusions (I)

- AtLYK3 is important for the cross-talk between ABA signaling and innate immunity.

-AtLYK3 may interact with other PRRs (CERK1?) and negatively regulate their activity in the absence of stimulus

Paparella, Savatin, Marti, De Lorenzo, Ferrari (2014) "The Arabidopsis LYSIN MOTIF-CONTAINING RECEPTOR-LIKE KINASE3 regulates the cross talk between immunity and abscisic acid responses". *Plant Physiology* 165(1):262-76. doi: 10.1104/pp.113.233759.

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