THE SAINSBURY LABORATORY

Plant-Microbe Interactions
-
An Introduction

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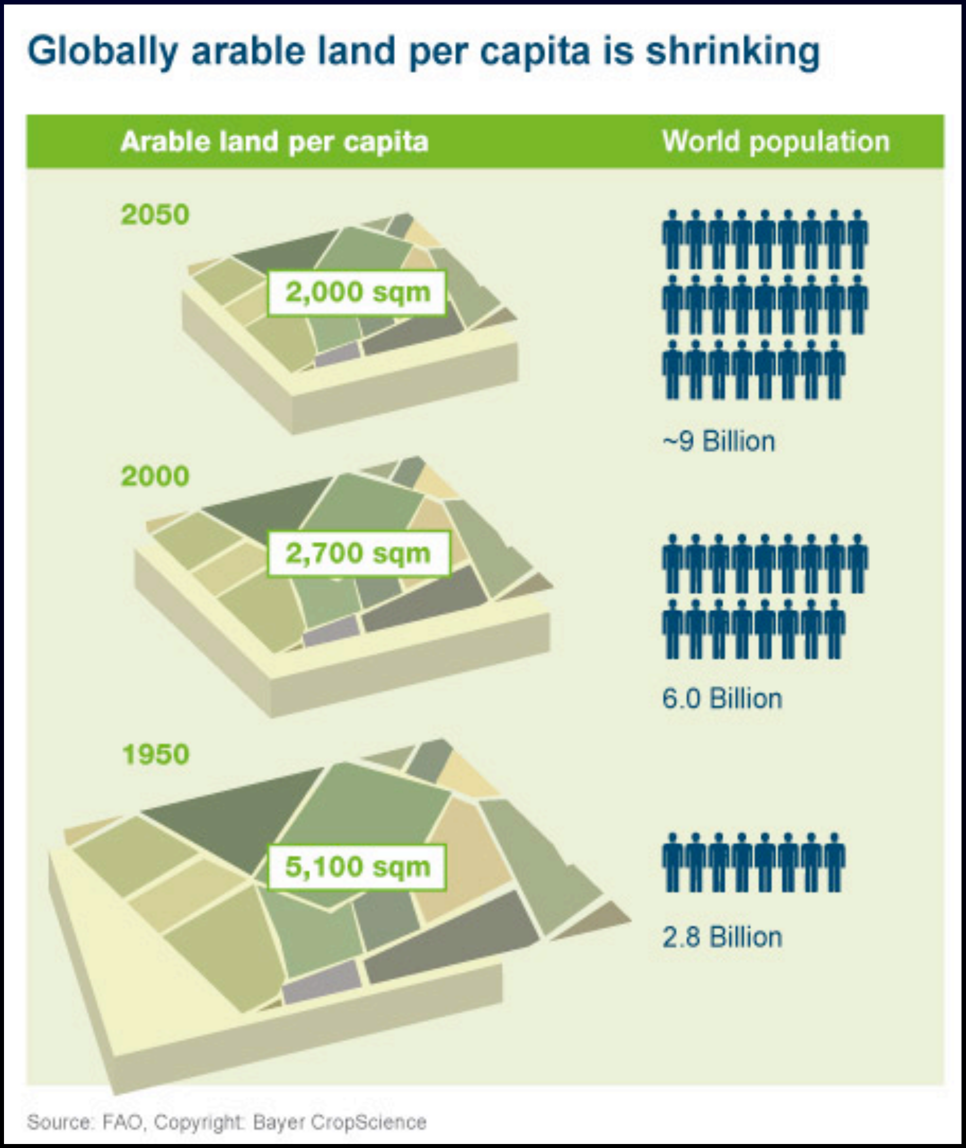
Plants are key to humanity

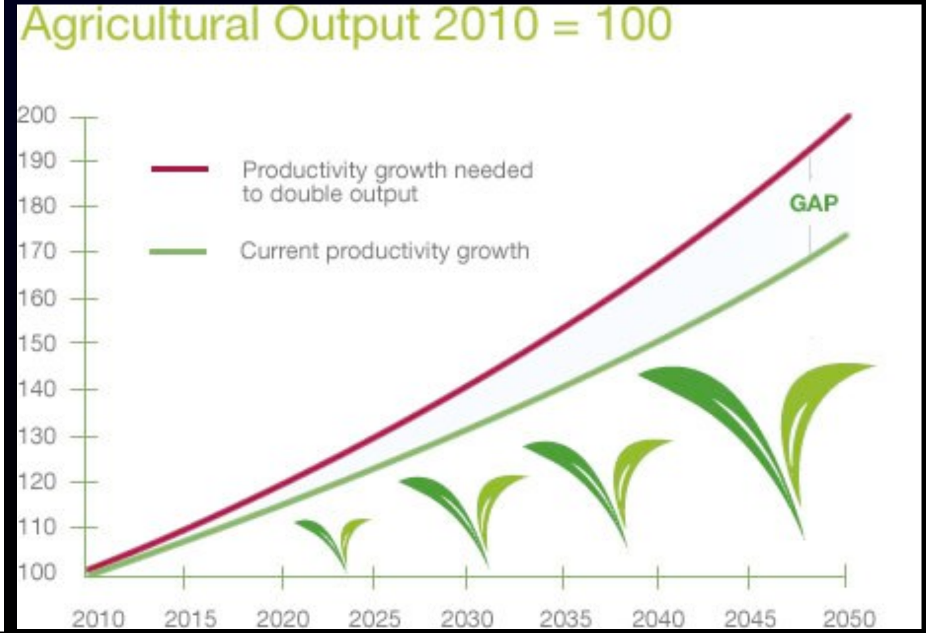
- Oxygen
- Food and drinks
- Materials
- Fuels
- Secondary metabolites (cosmetics, drugs, etc…)

Plant research as driver of major scientific discoveries – an immunity centred selection

- First genetic concepts
  Gregor Mendel (late 1800s), William Bateson (early 1900s)
- Discovery of transposable elements (40-50s)
  Barbara MacClintock, Nobel Prize 1983
- First surface immune receptors (PRRs) (1994/5)
  Jones et al., *Cell* 1994; Song et al., *Science* 1995
- First intracellular immune receptors (NLRs) (1994)
  Mindrinos et al., *Cell* 1994; Bent et al., *Science* 1994; Whitham et al., *Cell* 1994
- First identification of small RNAs (1999)
  Hamilton & Baulcombe, *Science* 1999
- First demonstration of gene silencing as anti-viral immune mechanism (1998/9)
  Brigneti et al., *EMBO J* 1998
- TAL effectors (2009)
  Boch et al., *Science* 2009; Moscou & Bogdanove, *Science* 2009
- Major concepts in host-microbe interactions (2000-present)

Food security: the global challenge of the 21st century





Plant diseases affect major crops worldwide

NEWS

Armed and Dangerous

These fungi, weeds, and viruses are among the more serious biological threats to food security—so researchers are working hard on countermeasures

WHEAT STEM RUST

Pest: *Puccinia graminis* Ug99
Crop: Wheat
Whereabouts: Fifty years ago, stem rust led to the resistant wheat varieties that fueled the Green Revolution—leading many farmers to believe they were done with *Puccinia graminis*. But in 1998, a dangerous new strain named Ug99 appeared in Uganda (*Science*, 30 March 2007, p. 1786). By 2004, its spread prompted Green Revolution pioneer Norman Borlaug to launch a global research initiative to address the threat. Ug99 has since shown up in Yemen and Iran and threatens wheat crops throughout the Middle East and West Asia. The big fear: Ug99 could cause famine in Pakistan and India, where small farmers can't afford the fungicides used to control the disease.
Symptoms: The fungus infiltrates stems and plugs up vascular tissue. Of the three common rust diseases, stem rust is the worst because it causes the plant to fall over, so the entire harvest is lost.
Losses: Heavy infections can reduce yields by 40% or more. If it reaches India's Punjab region, losses could reach \$3 billion per year; if it reaches the United States, the toll could be \$10 billion annually.
Countermeasures: The International Maize and Wheat Improvement Center in Mexico has created 15 resistant wheat varieties, but Ug99 is infamous for quickly overcoming resistance.

POTATO BLIGHT

Pest: *Phytophthora infestans*
Crops: Potatoes; also tomatoes and other solanaceous crops
Whereabouts: This funguslike organism occurs wherever farmers grow potatoes.
Symptoms: Most notorious for causing the Irish potato-famine of 1845 to 1851, late blight still ranks as the world's most dangerous potato disease. Spread by spores or by planting infected tubers, it first appears as gray splotches on leaves. In high humidity and moderate temperatures, it can destroy a whole field in a week.
Losses: The International Potato Center in Peru reports that yield losses in developing countries are about \$2.75 billion annually. Fungicide applications can total 10% of overall production costs.
Countermeasures: Fungicides work but can be harmful to human health and too costly for poor farmers.

BLACK SICATOKA

Pest: *Mycosphaerella fijiensis*
Crops: Bananas, plantains
Whereabouts: This fungus, first detected in Fiji in 1964, is now found in 100 countries in the Americas, Africa and South Asia.
Symptoms: The fungus starts as small flecks on the undersides of the youngest leaves. They expand into brown streaks that can eventually destroy the leaf, decreasing photosynthesis. Fruit from diseased trees can ripen prematurely during shipping, causing further losses.
Losses: Yields reduced up to 50%.
Countermeasures: Commercial plantations frequently apply cocktails of fungicides, sometimes from airplanes, and remove leaves at a cost of 15% to 50% of the fruit's final retail price.

CREDITS (TOP TO BOTTOM): E. R. FRENCH; CIP LIMA, BUGWOOD.ORG; (INSET) WIKIPEDIA; YUE JIN/USDA; FRED BROOKS, UNIVERSITY OF HAWAII AT MANOA, BUGWOOD.ORG

WITCHWEED

Pest: *Striga hermonthica*
Crops: Corn, sorghum, sugarcane, millet, native grasses
Whereabouts: *Striga* originated in Africa and has since become widespread in the tropics.
Symptoms: This parasitic plant attaches to the host's roots, where it siphons off nutrients and water, stunting the host's growth and causing it to wither. When *Striga* emerges aboveground, it makes a substance toxic to the host. One plant can produce 50,000 tiny seeds that stick to people and their tools or settle in the soil. Seeds can stay dormant for 15 years.
Losses: In sub-Saharan Africa, *Striga* infects 20 million to 40 million hectares, reducing yields by 20% to 100%. Losses total about \$1 billion per year and affect 100 million people.
Countermeasures: Some *Striga*-tolerant maize can produce small ears despite being parasitized. But farmers must scramble to destroy plants before they produce seed and plant nonhost crops in affected soils. Another approach is to plant a legume called *Desmodium*, which secretes a chemical that kills *Striga*, but that requires using livestock to control the *Desmodium*. Researchers are looking into applying a fungus to kill the seeds.

SPECIALSECTION

ASIAN SOYBEAN RUST

Pest: *Phakopsora pachyrhizi*
Crops: At least 31 legume species, notably soybeans
Whereabouts: Native to Asia, soybean rust spread to Australia in the 1980s and reached Africa a decade later. It hit South America in 2001, and Hurricane Ivan carried spores into the United States in 2004. It's now found throughout the Southeastern United States and Mexico (*Science*, 3 December 2004, p. 1672).
Symptoms: Infected plants develop small pustules on the undersides of leaves that spread throughout the plant. In the United States, the invasive vine kudzu is the primary host and vector for soybean rust.
Losses: Yields reduced 10% to 80%.
Countermeasures: Early detection and multiple applications of fungicide.

RICE BLAST

Pest: *Magnaporthe oryzae*
Crops: Rice, 50 species of grasses and sedges
Whereabouts: Worldwide
Symptoms: Spores infect plants, particularly when humidity is high, often killing young plants. In older plants, the fungus can spread and prevent seed formation.
Losses: Destruction can be extremely fast but variable, with up to 100% loss in some paddies. Some analysts estimate that each year blast destroys harvests that could feed 60 million people, at a cost of some \$66 billion.
Countermeasures: Rice blast is a formidable foe, persisting despite the best control efforts. Farmers can manage the disease by rotating crops, maintaining water levels (too little water promotes infection), and using fertilizers prudently. Resistant cultivars help, but no cultivar can withstand all races of the fungus, and blast tends to overcome resistance in two or three growing seasons. Farmers can also use fungicides.

CASSAVA BROWN STREAK VIRUS

Pest: Virus
Crops: Cassava, also called yucca, manioc, and mandioca
Whereabouts: East and Central Africa
Symptoms: This virus is emerging as a major threat to a crop already under siege from cassava mosaic virus. Spread by whiteflies and by cuttings, brown streak virus is more insidious than the mosaic virus because the plant can look healthy even as the disease destroys the edible root. Once confined to lowlands in East Africa, it appeared in Uganda in 2004 and has become a threat throughout sub-Saharan Africa. Disease often appears where farmers have planted cassava varieties resistant to mosaic virus.
Losses: Yields drop by up to 100%. In 2003, economic losses totaled more than \$100 million per year. This virus and cassava mosaic virus have been called Africa's biggest threat to food security.
Countermeasures: The International Institute of Tropical Agriculture, based in Nigeria, is developing tolerant varieties whose leaves become diseased but whose roots stay healthy. Early-warning monitoring programs and early harvesting can help reduce the impact of the diseases.

–ELIZABETH PENNISI

CREDITS (TOP TO BOTTOM): USDA APHIS PPQ, BUGWOOD.ORG; CHRISTINE STONE/USDA; IITA; DONALD GROTH, LSU AGCENTER, BUGWOOD.ORG

Global intensification of agriculture

Global intensification of agriculture… and plant diseases

Global impact of plant pathogens

(+ OOMYCETES…)

Fisher *et al*. 2012

Crop *Host species*	2009/2010 harvest (million tonnes)	Calories per 100g flour (un-cooked)	Disease/Pathogen and variation in % losses	Loss of food* for *x* million over 1 year, given diet of 2,000 calories per day
Rice	701 harvest but	325	Rice Blast	212 to 742
			10-80%	
				TOTAL: Could feed 596 – 4,287 million mouths *per annum***

TOTAL: Could feed 596 – 4,287 million mouths *per annum***

We need to study the plants' own immune system to understand how pathogens cause diseases, and how to fight them

Plant-microbe interactions: from mechanistic understanding to use

Science **2013**

REVIEW

Pivoting the Plant Immune System from Dissection to Deployment

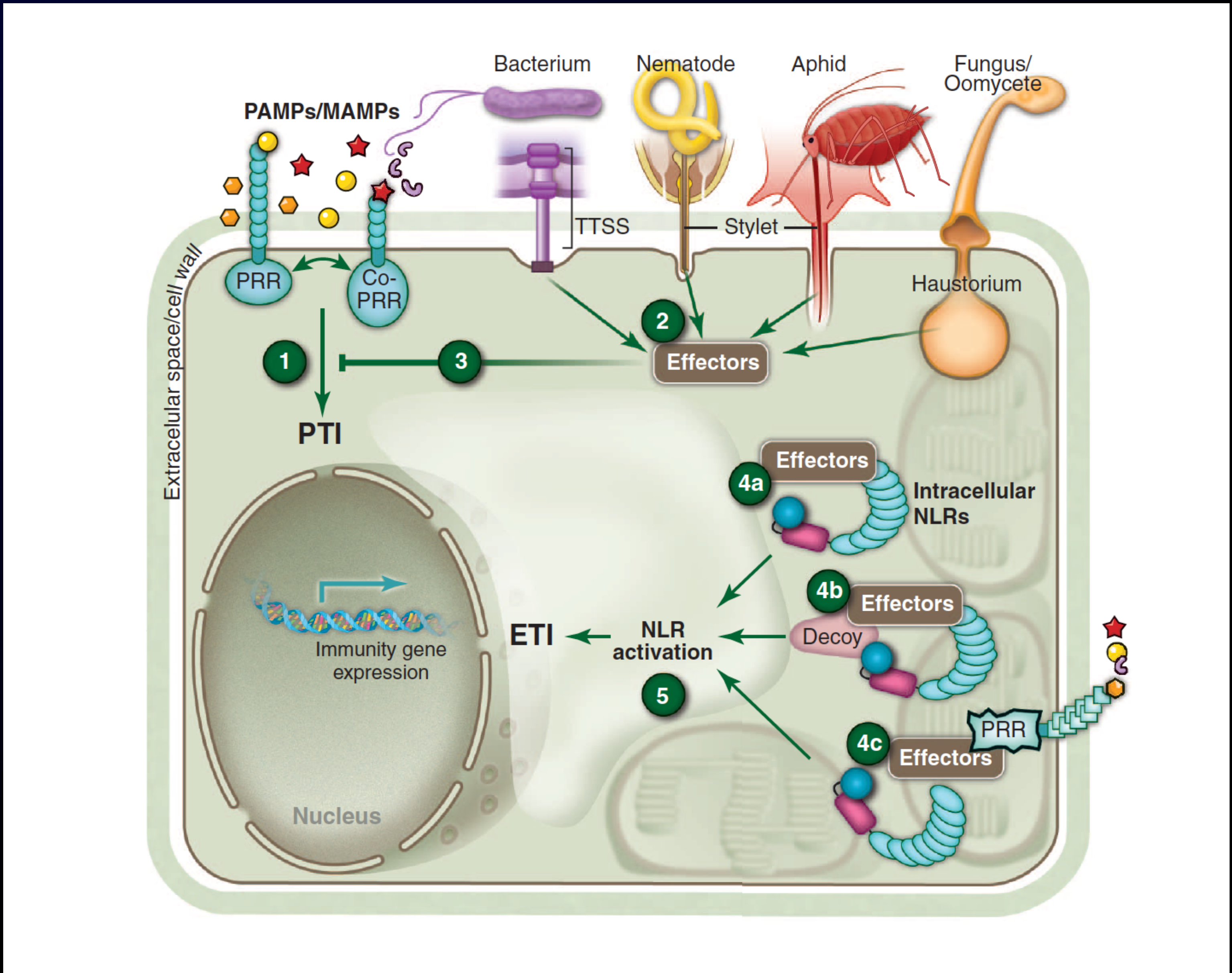
Jeffery L. Dangl,[1,2,3,4,5*†] Diana M. Horvath,[6*] Brian J. Staskawicz[7*]

Diverse and rapidly evolving pathogens cause plant diseases and epidemics that threaten crop yield and food security around the world. Research over the last 25 years has led to an increasingly clear conceptual understanding of the molecular components of the plant immune system. Combined with ever-cheaper DNA-sequencing technology and the rich diversity of germ plasm manipulated for over a century by plant breeders, we now have the means to begin development of durable (long-lasting) disease resistance beyond the limits imposed by conventional breeding and in a manner that will replace costly and unsustainable chemical controls.

Plant immunity

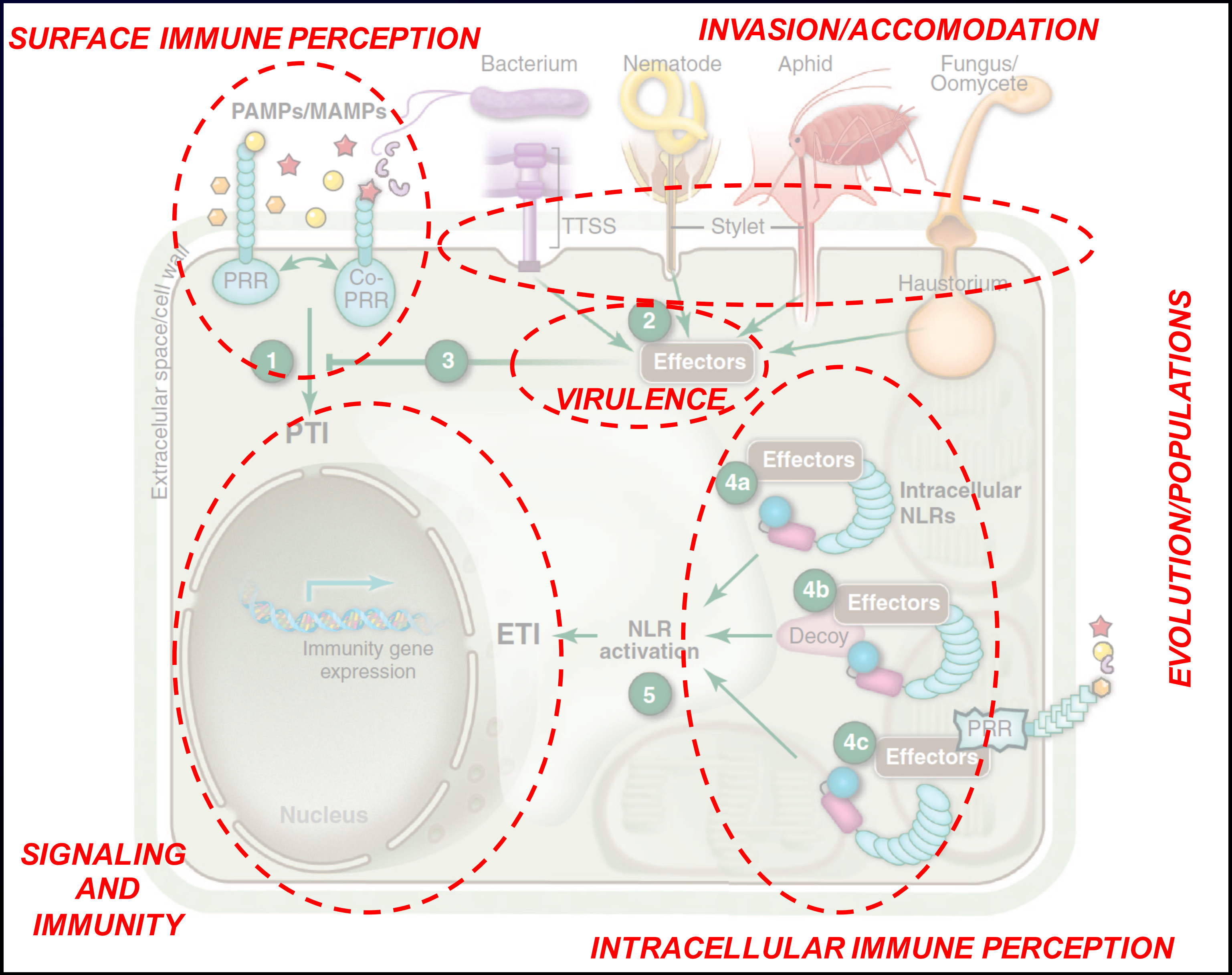
- All plant immune responses are innate
- No specialized circulatory immune cells
- Both local and systemic immune responses
- Transgenerational ‘memory’ of stress(?)

A modern synthesis of plant innate immunity



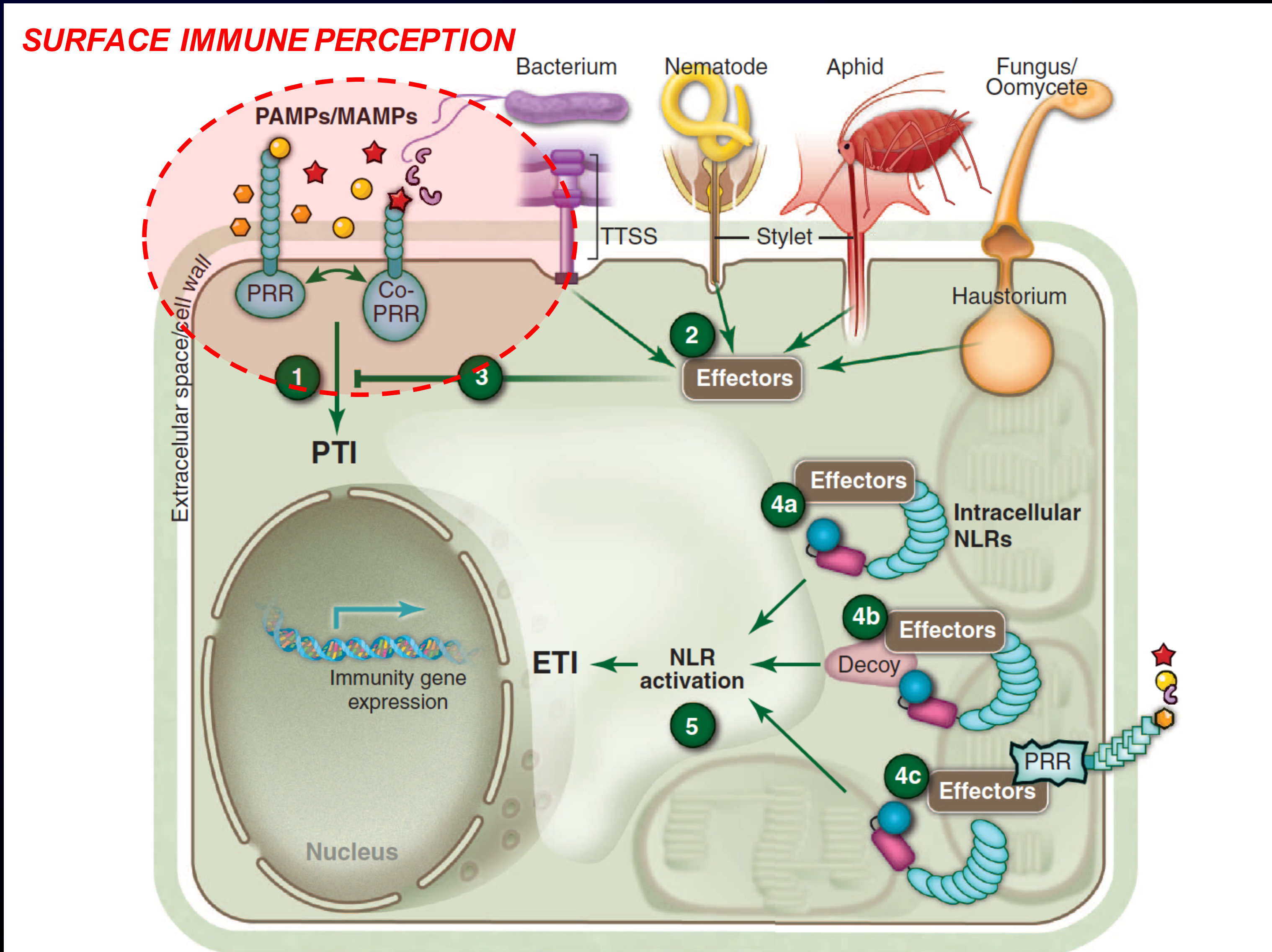
Dangl et *al.*, *Science* 2013

Deciphering plant innate immunity and its interaction with microbes



Adapted from Dangl et *al.*, *Science* 2013

PRR-triggered immunity (PTI) or Surface Immunity



PRR: pattern recognition receptor
PAMP/MAMP: pathogen/microbe-associated molecular pattern

Adapted from Dangl et *al., Science* 2013

Plants recognize a number of ‘non-self’/’altered-self’ molecules

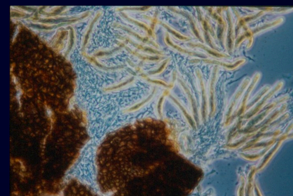
PAMPs recognized in the plant model *Arabidopsis thaliana*

Oomycetes

- glucans
- Pep13
- INF1
- NPP1
- PaNie
- CBEL

…

Fungi



- xylanase
- chitin
- ergosterol

…

Bacteria

- flagellin (flg22)
- EF-Tu (elf18)
- lipopolysaccharides (LPS)
- peptidoglycans (PGN)
- cold-shock protein (csp22)
- RaxX

…

Viruses

- dsRNAs

…

Insects
Nematodes
(*e.g.* ascarosides)

Parasitic plants
(*e.g.* glycosylated peptide)

plant cell

‘danger’ signals

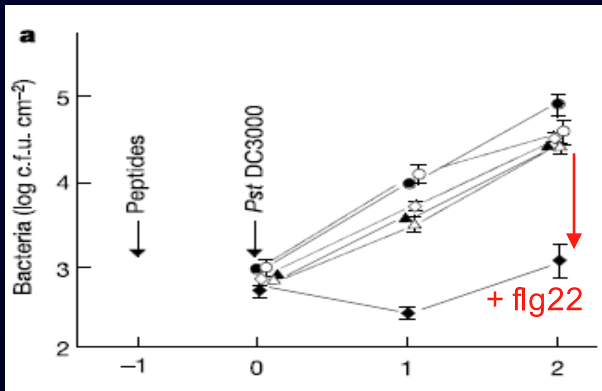
- oligogalacturonides
- AtPep1
- eATP

…

<u>Note</u>: not all PAMPs are recognized by all plant species

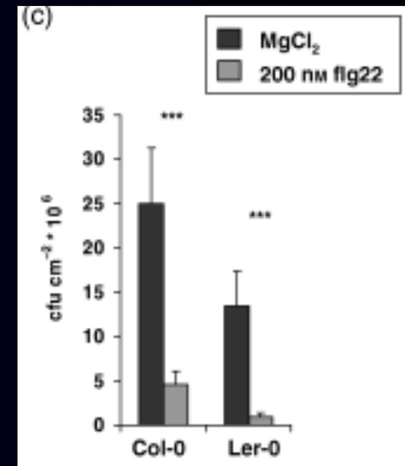
PAMP-induced resistance: broad-spectrum, local and systemic

Pseudomonas syringae

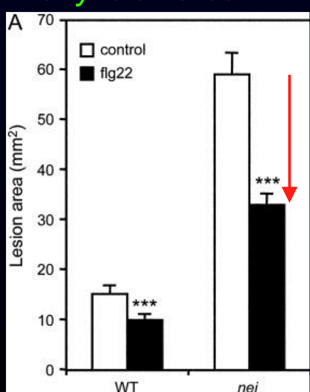


Zipfel et *al.*, *Nature* 2004

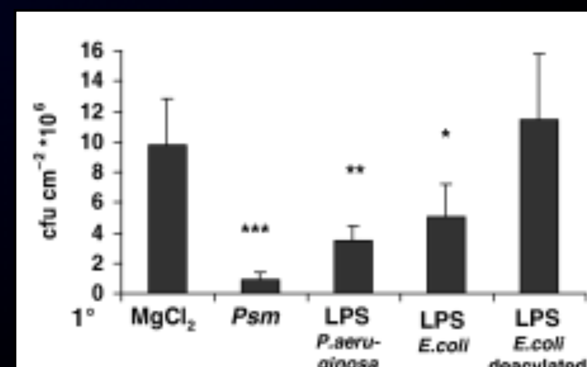
SAR against *Pseudomonas syringae*



Botrytis cinerea

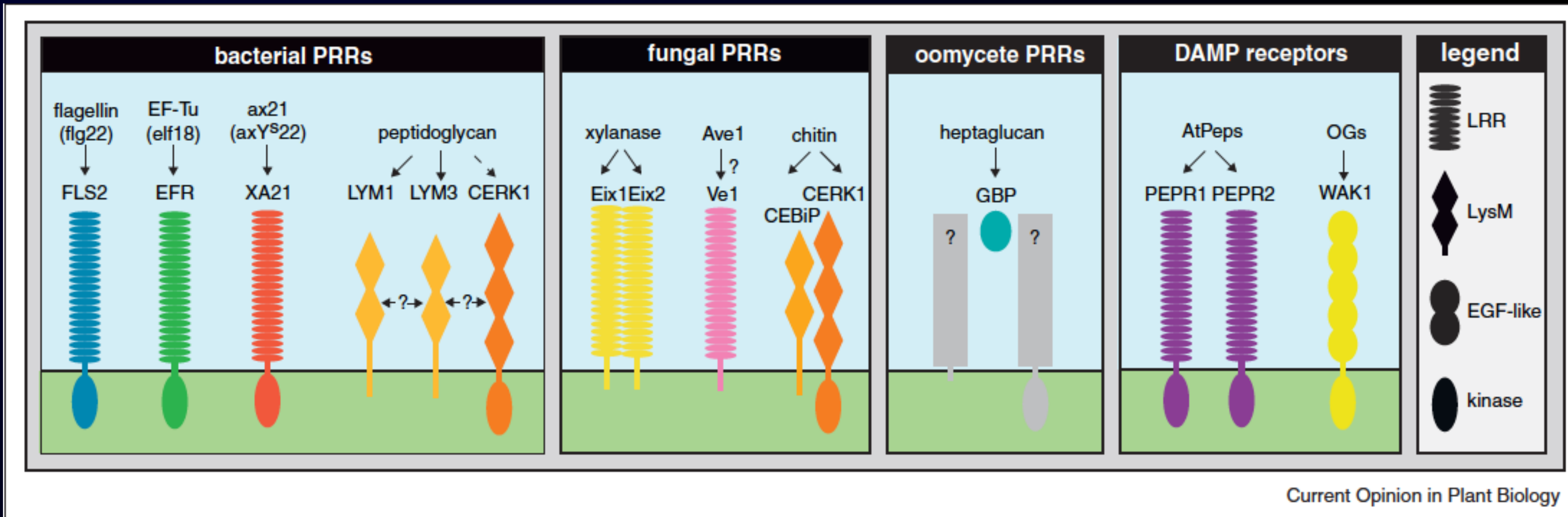


Ferrari et *al.*, *Plant Physiology* 2007



Mishina & Zeier, *Plant J* 2007

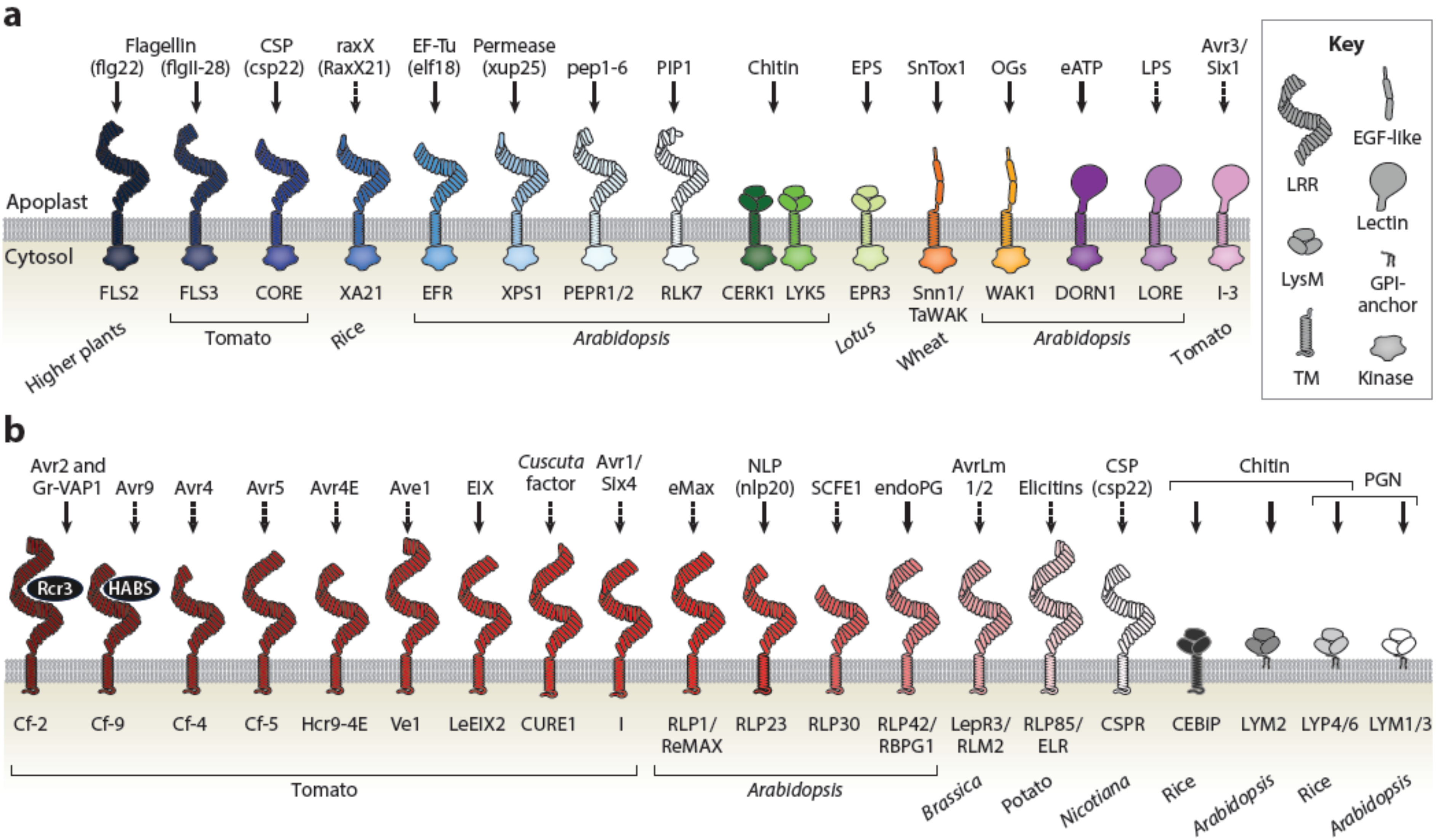
Plant PRRs are either receptor kinases or receptor-like proteins



Monaghan & Zipfel, *COPB* 2012

- PRRs contribute to both basal and non-host resistances
- PAMP confers broad-spectrum disease resistance
- Only a few plant PRRs currently know…

An increasing number of known plant PRRs



Boutrot & Zipfel, *Ann. Rev. Phytopath.*, 2017

Examples of PRRs that underlie disease resistance in crops

A Receptor Kinase–Like Protein Encoded by the Rice Disease Resistance Gene, *Xa21*

SCIENCE • VOL. 270 • 15 DECEMBER 1995

Wen-Yuan Song,* Guo-Liang Wang, Li-Li Chen, Han-Suk Kim, Li-Ya Pi, Tom Holsten, J. Gardner, Bei Wang,† Wen-Xue Zhai, Li-Huang Zhu, Claude Fauquet, Pamela Ronald‡

The Plant Journal (2006) **46**, 794–804 doi: 10.1111/j.1365-313X.2006.02739.x

A B-lectin receptor kinase gene conferring rice blast resistance

Xuewei Chen[1,†,‡], Junjun Shang[1,4,‡], Dexi Chen[2], Cailin Lei[3], Yan Zou[1,4], Wenxue Zhai[1], Guozhen Liu[1], Jichen Xu[1], Zhongzhuan Ling[3], Gang Cao[2], Bingtian Ma[2], Yuping Wang[2], Xianfeng Zhao[1], Shigui Li[2,*] and Lihuang Zhu[1,*]

[1]*Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China,*
[2]*Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, China*
[3]*Institute of Crop Research, Chinese Academy of Agricultural Sciences, Beijing 100081, China, and*
[4]*Graduate School of Chinese Academy of Sciences, Beijing 100101, China*

A maize wall-associated kinase confers quantitative resistance to head smut

NATURE GENETICS VOLUME 47 | NUMBER 2 | FEBRUARY 2015

Weiliang Zuo[1,5], Qing Chao[1,5], Nan Zhang[1,5], Jianrong Ye[1], Guoqing Tan[2], Bailin Li[3], Yuexian Xing[2], Boqi Zhang[1], Haijun Liu[4], Kevin A Fengler[3], Jing Zhao[1], Xianrong Zhao[1], Yongsheng Chen[1], Jinsheng Lai[1], Jianbing Yan[4] & Mingliang Xu[1]

A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice

NATURE BIOTECHNOLOGY VOLUME 33 NUMBER 3 MARCH 2015

Yuqiang Liu[1,3], Han Wu[1,3], Hong Chen[1], Yanling Liu[1], Jun He[1], Haiyan Kang[1], Zhiguang Sun[1], Gen Pan[1], Qi Wang[1], Jinlong Hu[1], Feng Zhou[1], Kunneng Zhou[1], Xiaoming Zheng[2], Yulong Ren[1], Liangming Chen[1], Yihua Wang[1], Zhigang Zhao[1], Qibing Lin[2], Fuqing Wu[2], Xin Zhang[2], Xiuping Guo[2], Xianian Cheng[1], Ling Jiang[1], Chuanyin Wu[2], Haiyang Wang[2] & Jianmin Wan[1,2]

PNAS

The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase

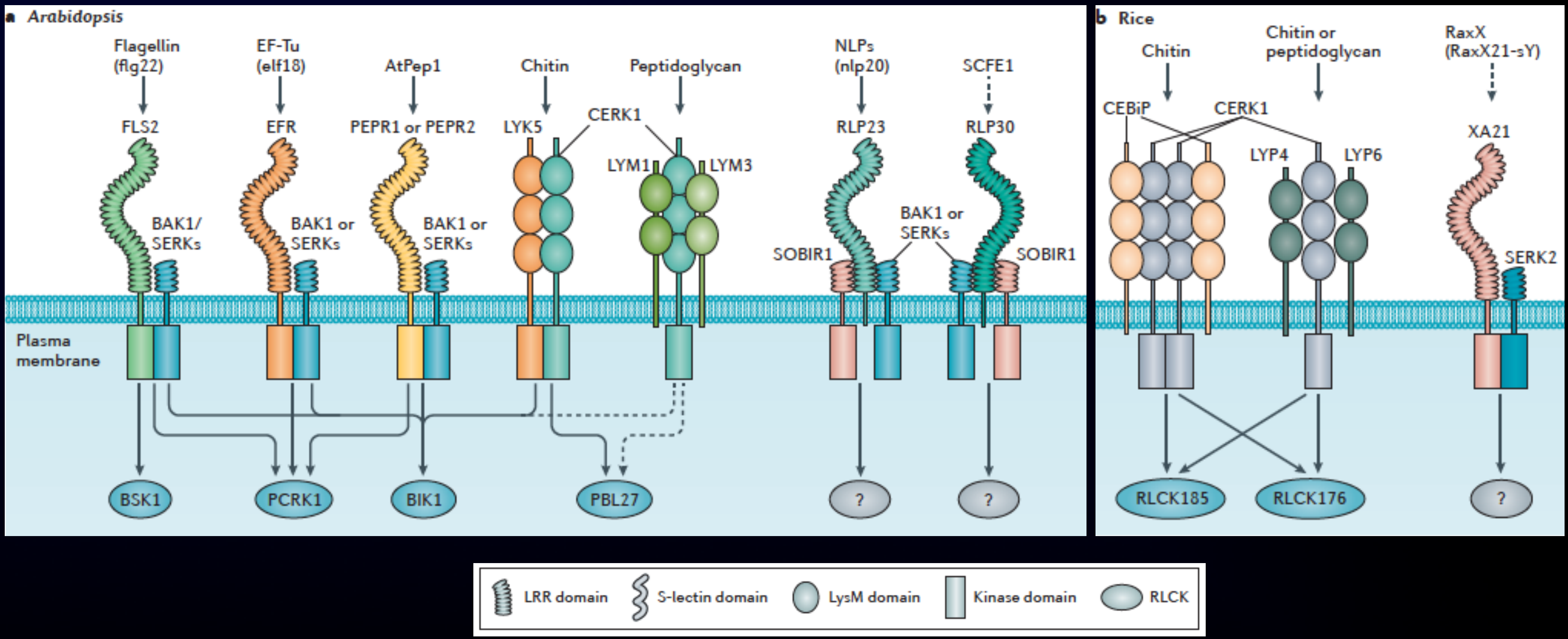
Severine Hurni[a,1], Daniela Scheuermann[b,1], Simon G. Krattinger[a,1], Bettina Kessel[b,1], Thomas Wicker[a], Gerhard Herren[a], Mirjam N. Fitze[a], James Breen[a,2], Thomas Presterl[b], Milena Ouzunova[b,3], and Beat Keller[a,3]

[a]Institute of Plant Biology, University of Zurich, CH-8008 Zurich, Switzerland; and [b]KWS SAAT SE, DE-37574 Einbeck, Germany

… but no corresponding ligand known yet…

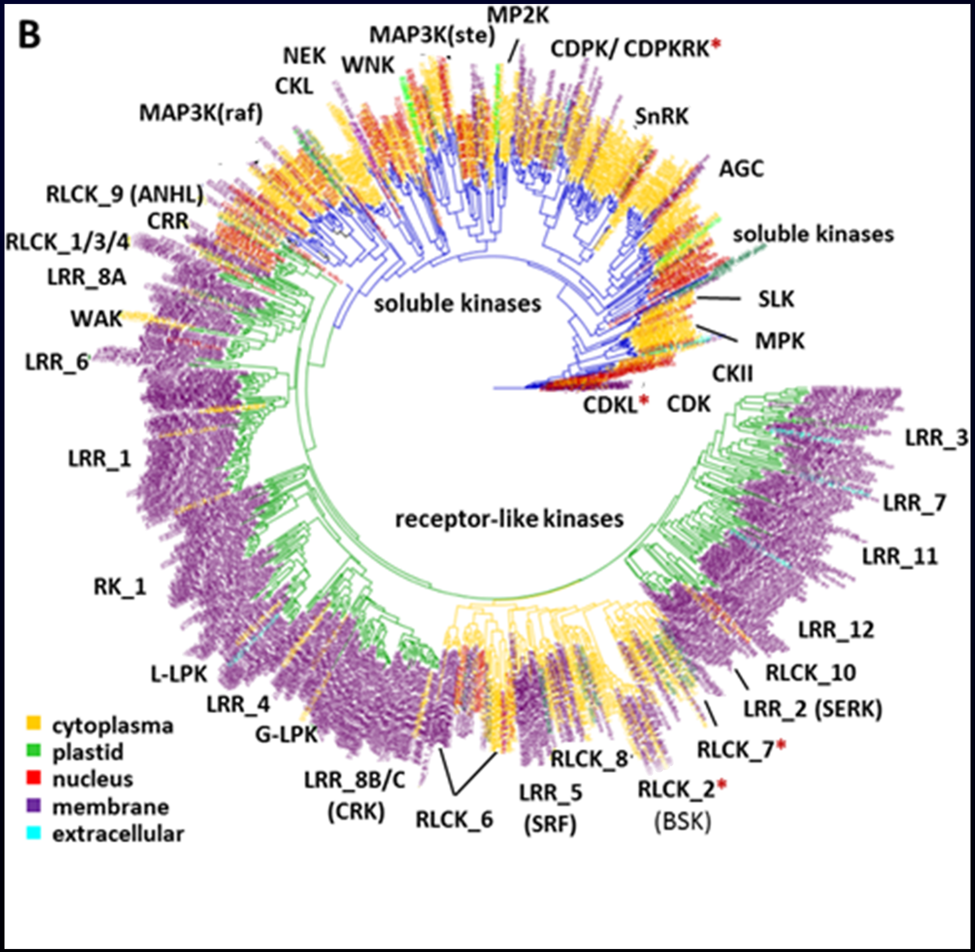
PRRs work as part of heteromeric protein complexes

Some RKs are co-receptors or regulatory RKs for ligand-binding PRRs (RKs or RLPs)

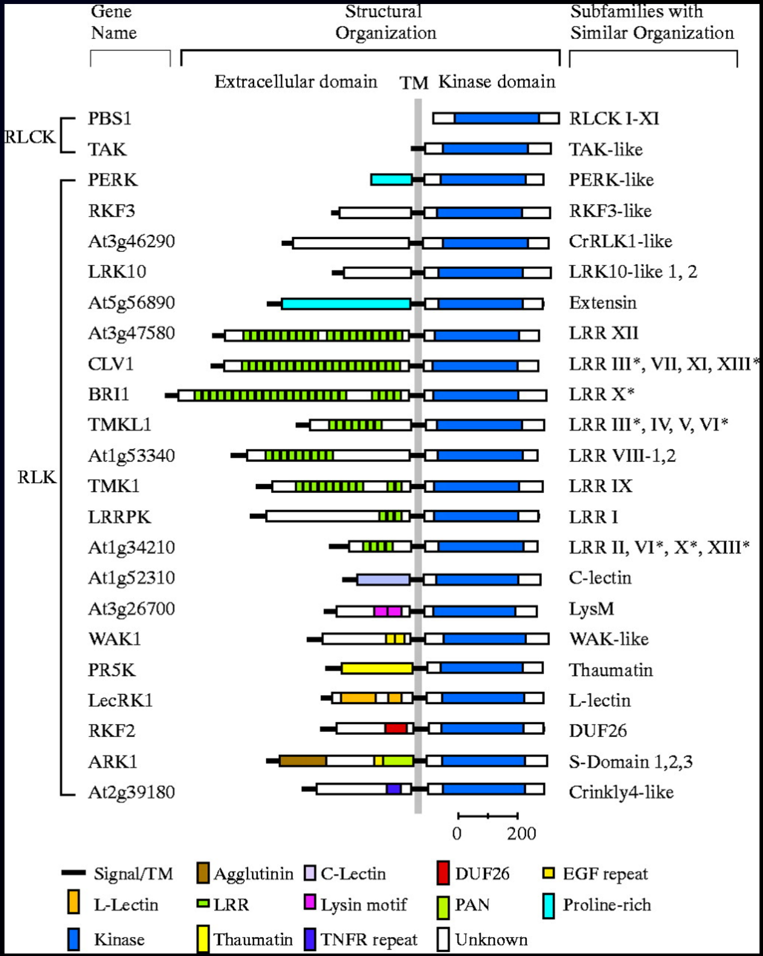


Couto & Zipfel, *Nature Rev. Immunol., 2016*

Plant genomes encode hundreds of potential PRRs

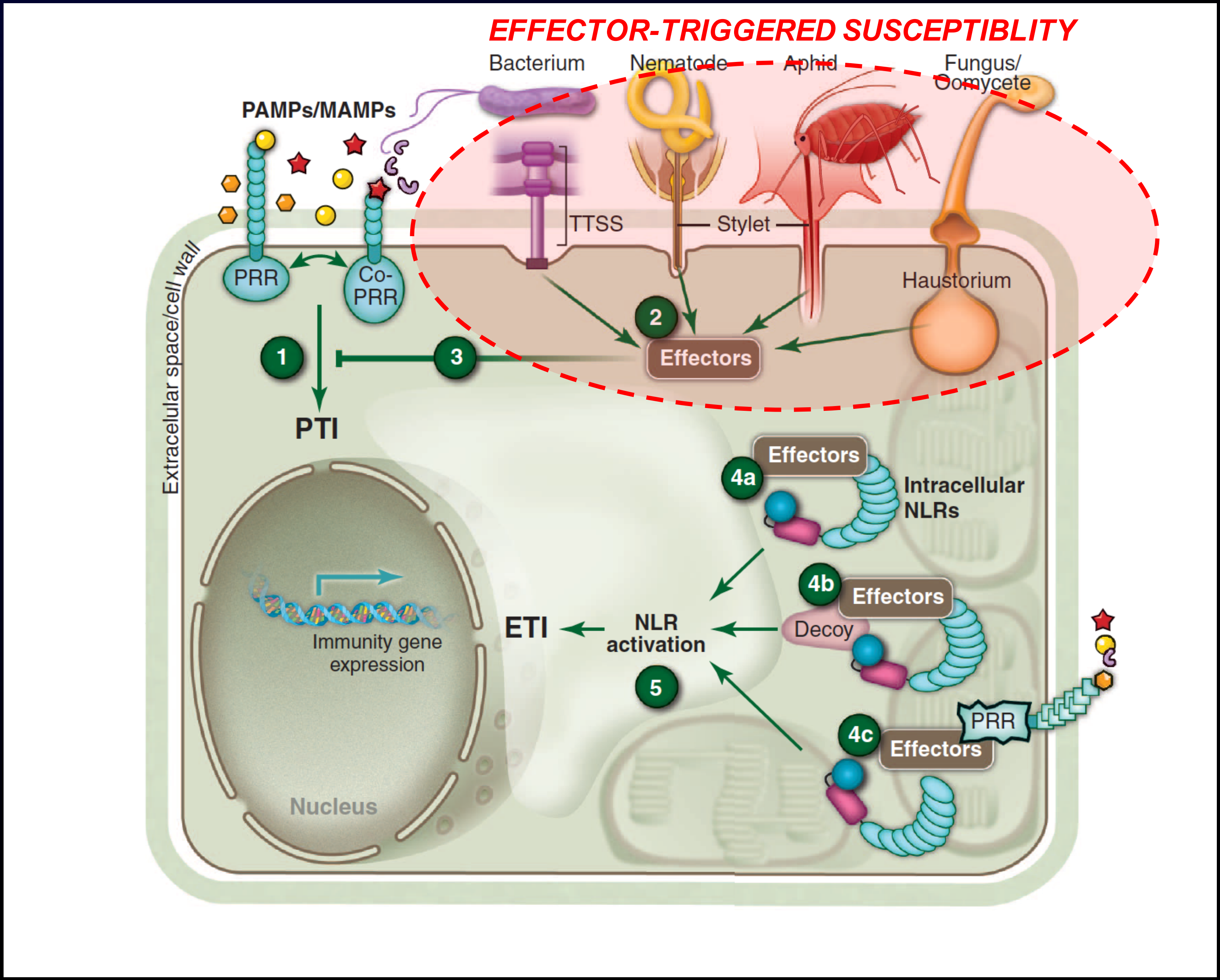


Zulawski et al., *BMC Genomics* 2014



Shiu & Bleecker, *PNAS* 2001

Effector-triggered susceptibility (ETS)



Adapted from Dangl et *al., Science* 2013

Effectors are remarkable pathogen molecules that act in the host

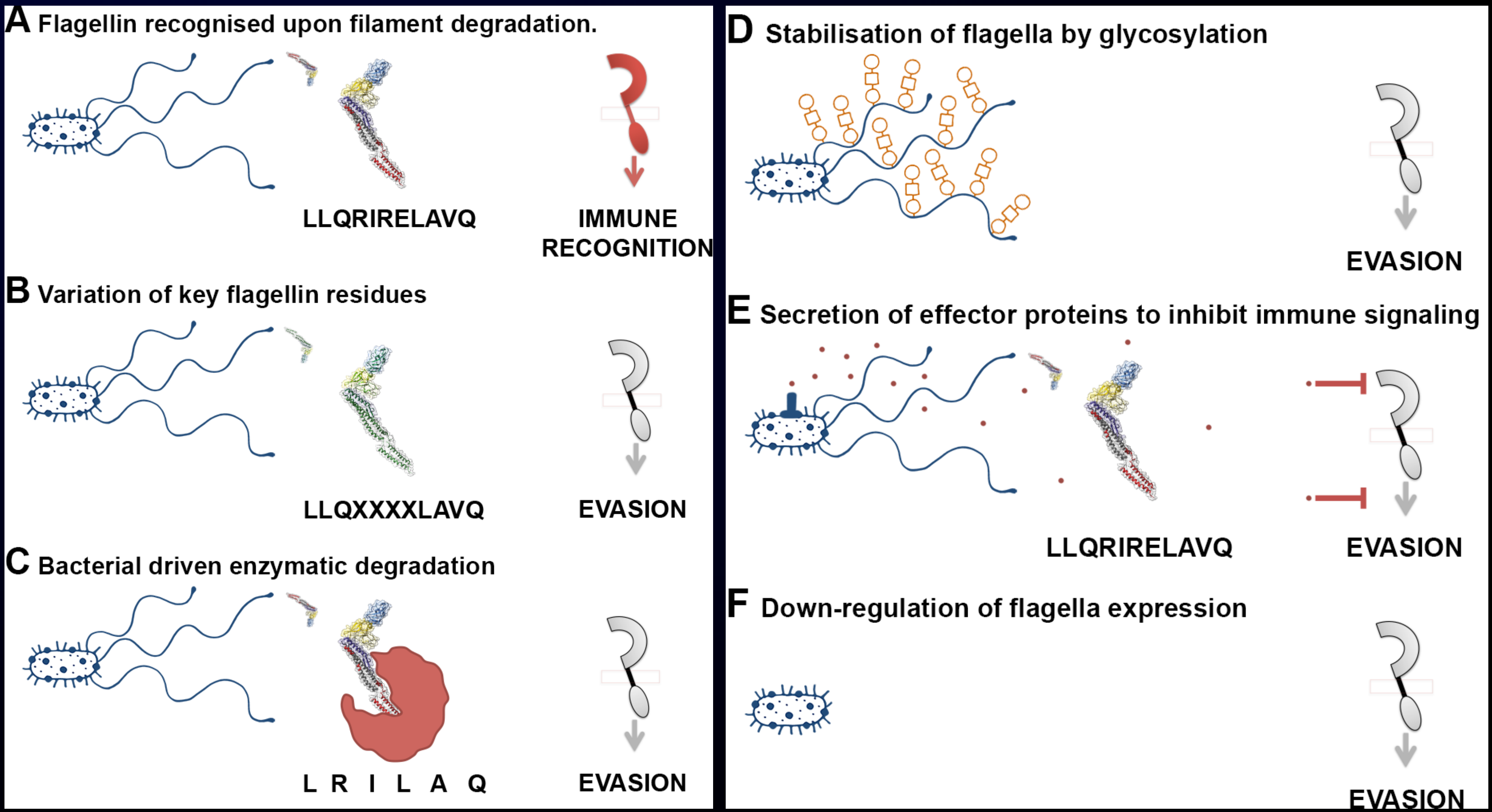
- Effectors are *de facto* ‘plant molecules’ (concept of ‘extended phenotype’).

- Effectors are molecules that affect the host physiology for the pathogen’s benefit.

- Diversifying selection caused by arms race with hosts.

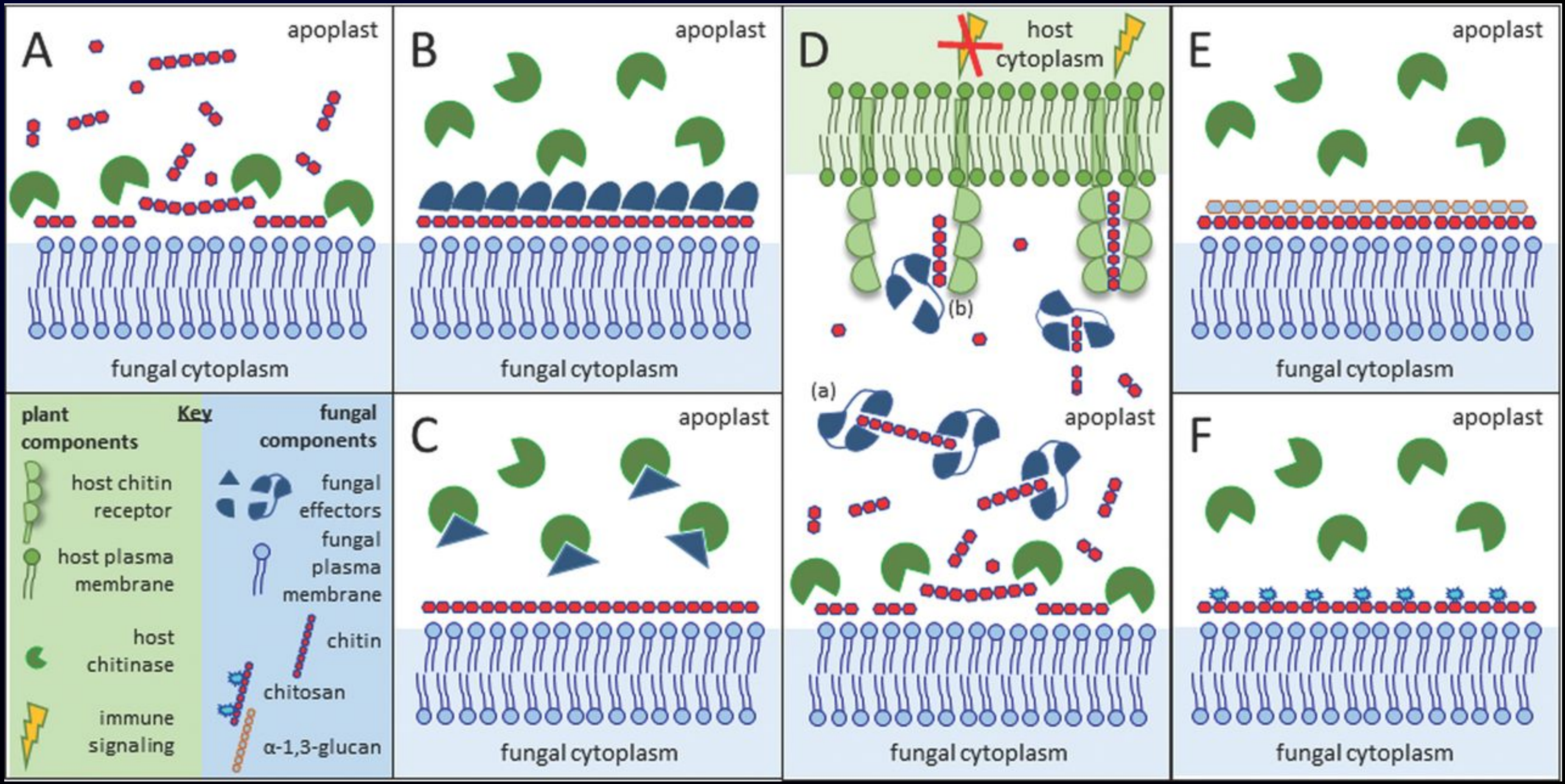
- The study of effectors tells us what is actually relevant in the context of a particular plant-microbe interaction.

Successful pathogens must evade or suppress PTI: *e.g.* flagellin



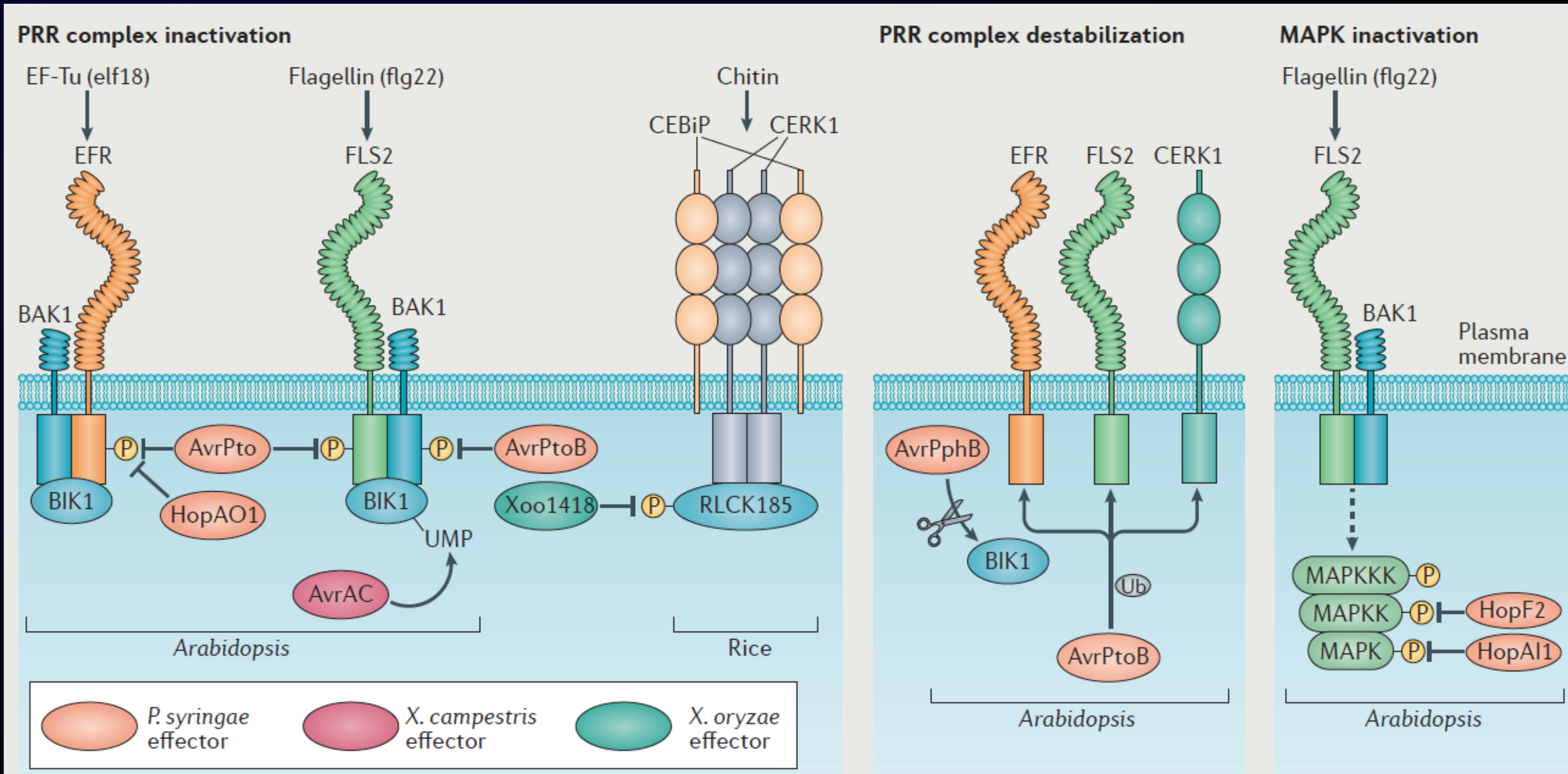
Rossez et al. *PLoS Pathogens* 2015

Successful pathogens must evade or suppress PTI: *e.g.* chitin



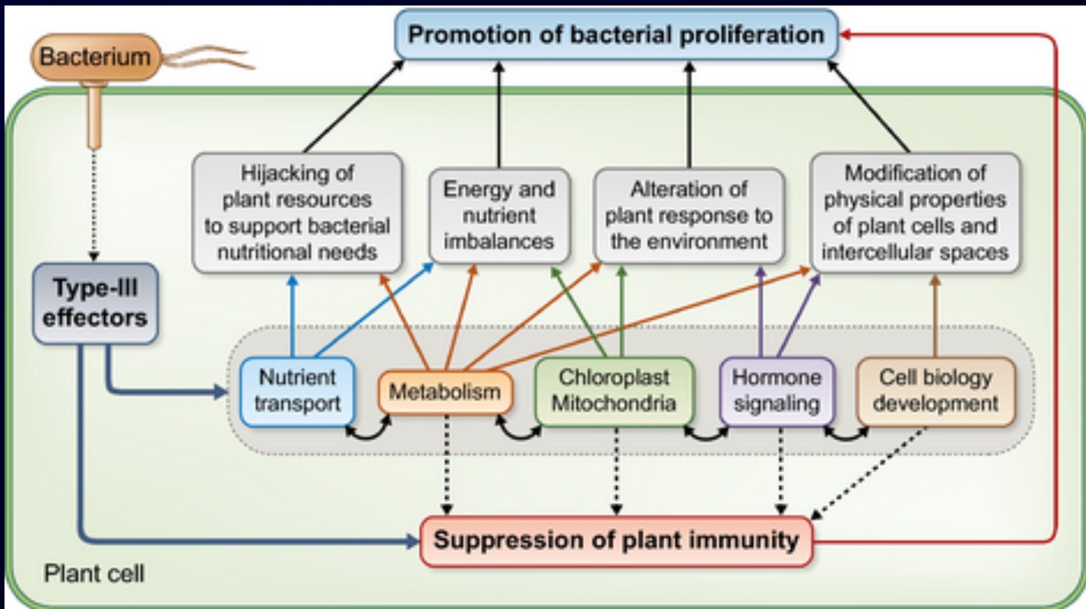
Sánchez-Vallet et al. *FEMS Microbiol Rev* 2015

Suppression of PTI by bacterial type 3-secreted effectors

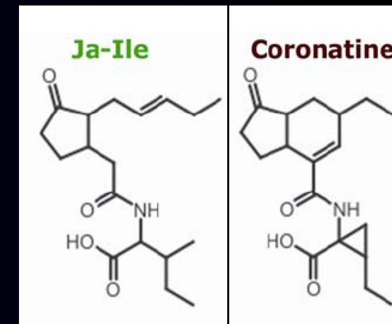


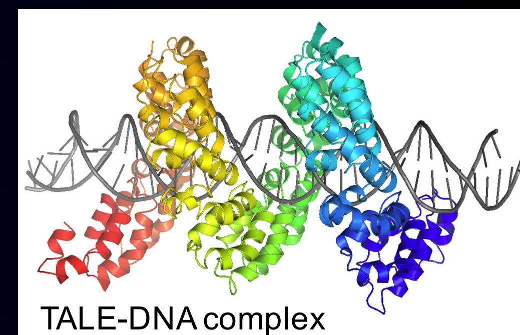
Couto & Zipfel, *Nature Rev. Immunol.*, 2016

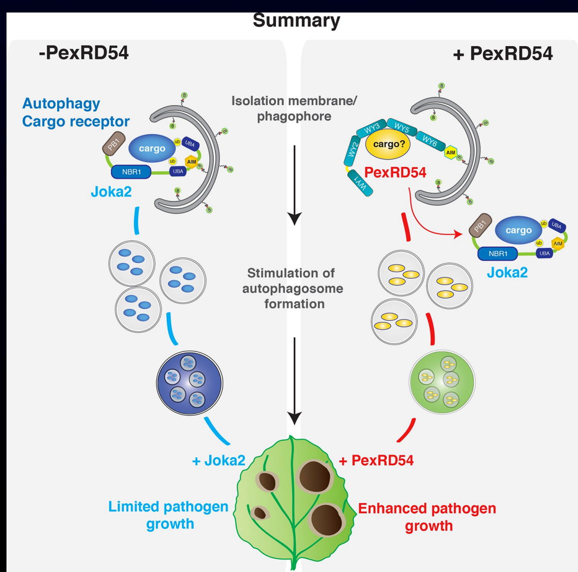
Effectors: beyond PTI suppression

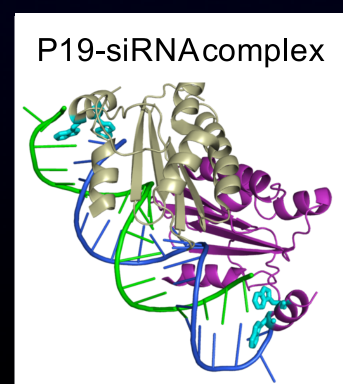


Macho, New Phytol., *2016*



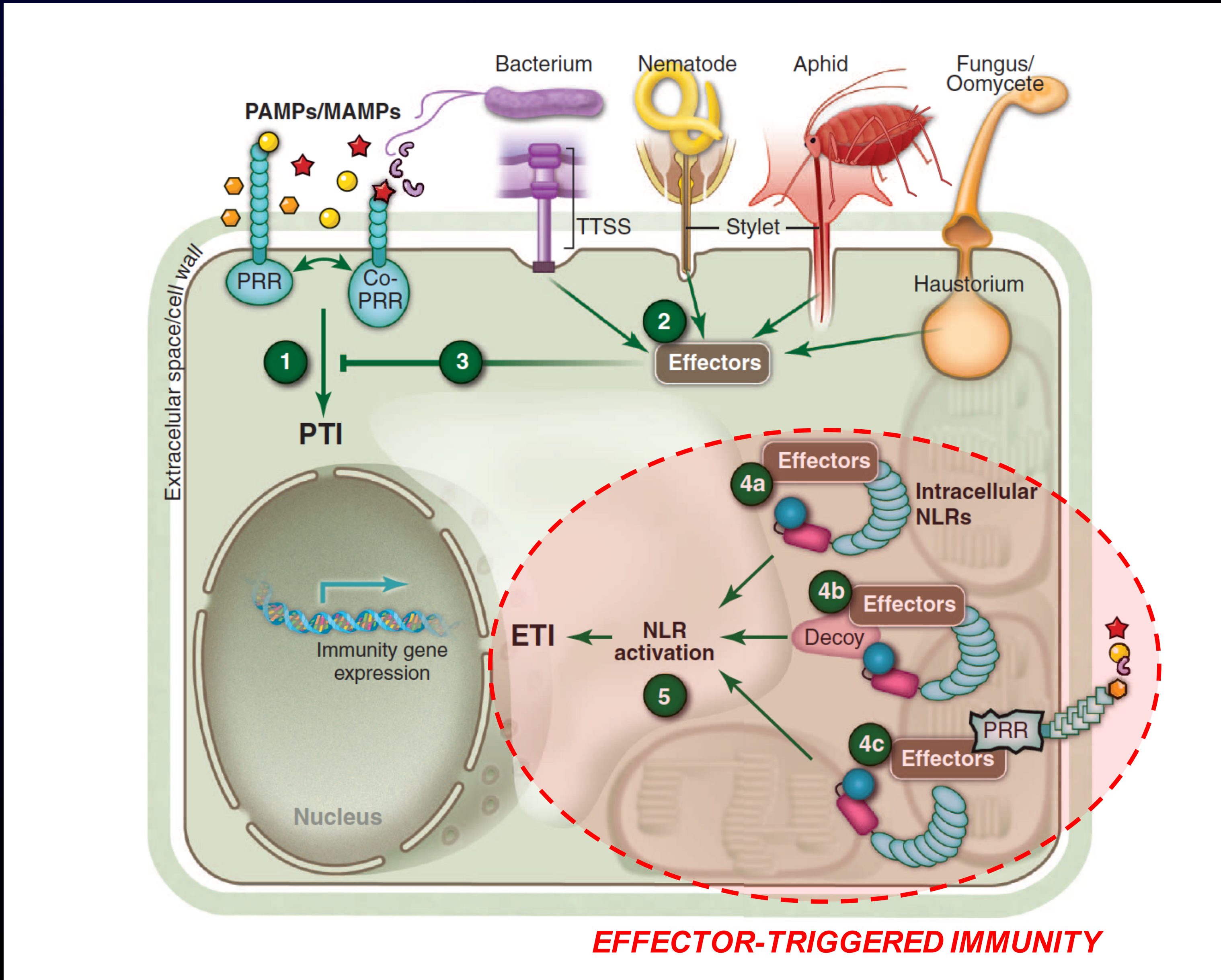






phytoplasma SAP54

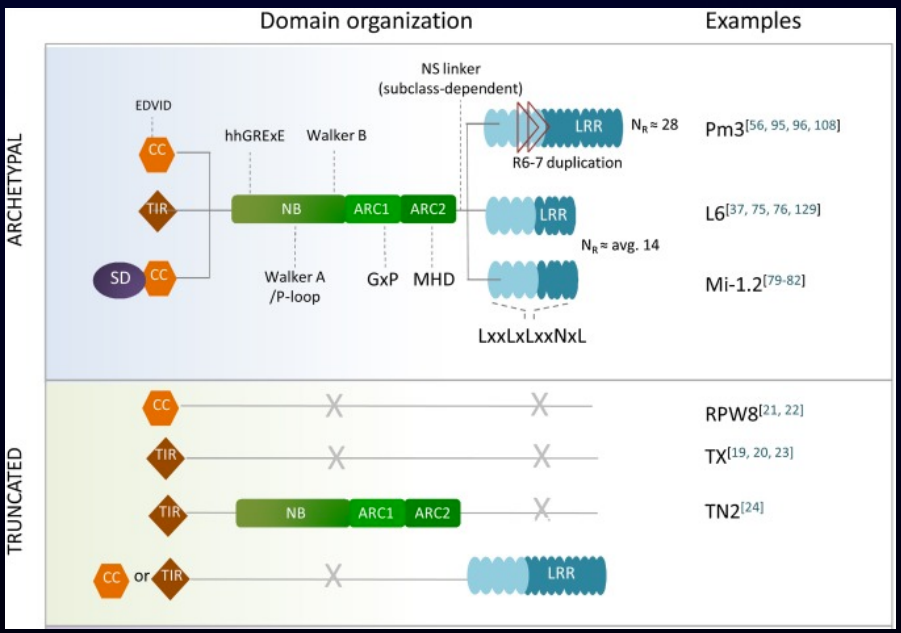
Effector-triggered immunity (ETI) or Intracellular Immunity

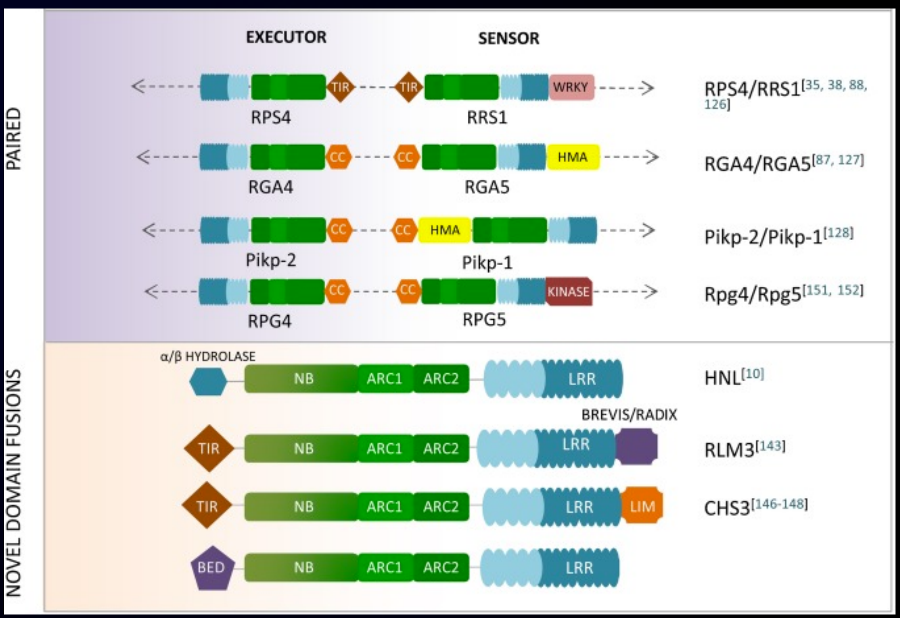


Adapted from Dangl et *al., Science* 2013

Different flavours of plant intracellular immune receptors (NLRs)

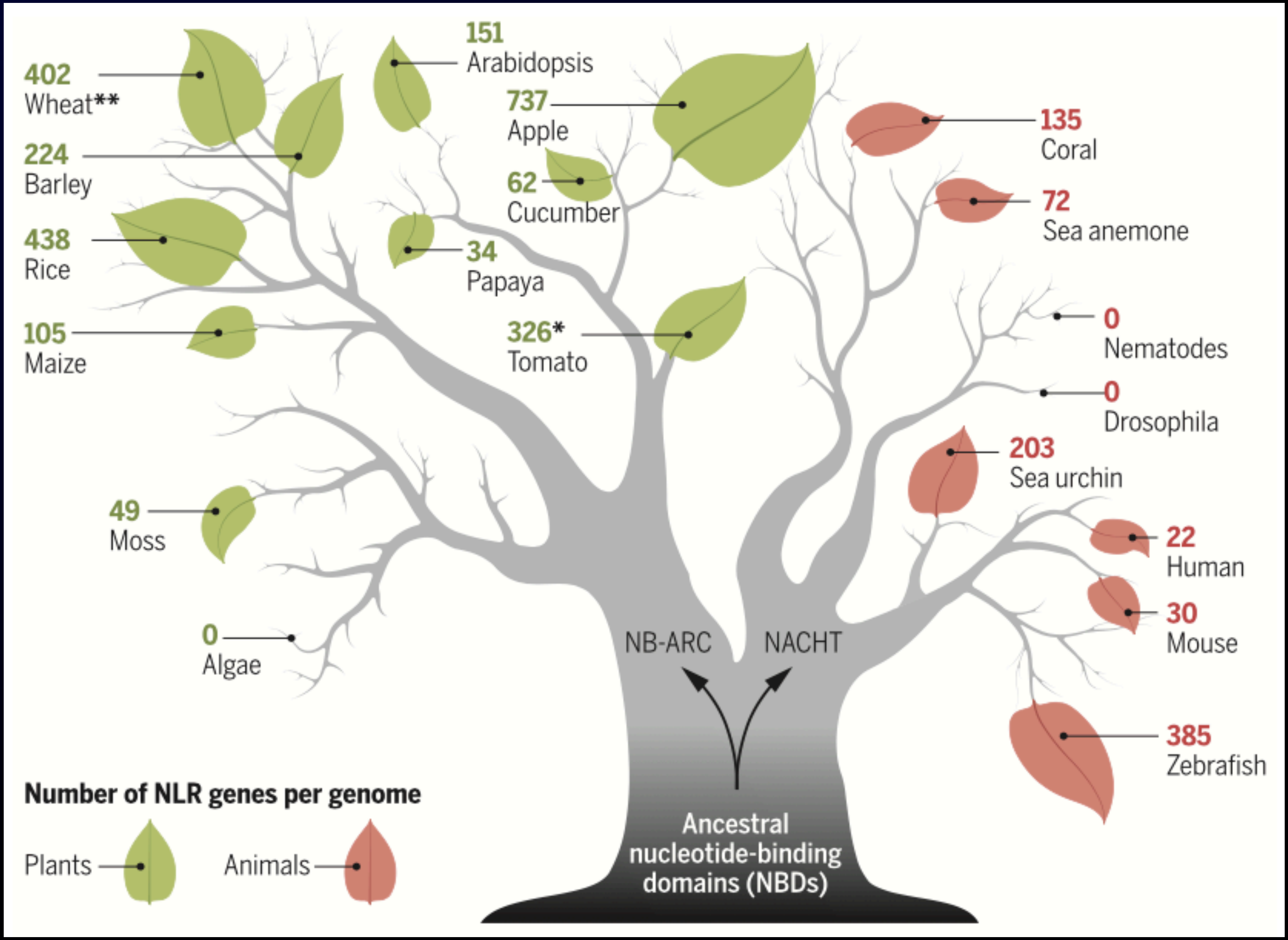
NLR = nucleotide binding-leucine-rich repeat receptor
(or NOD-like receptor)





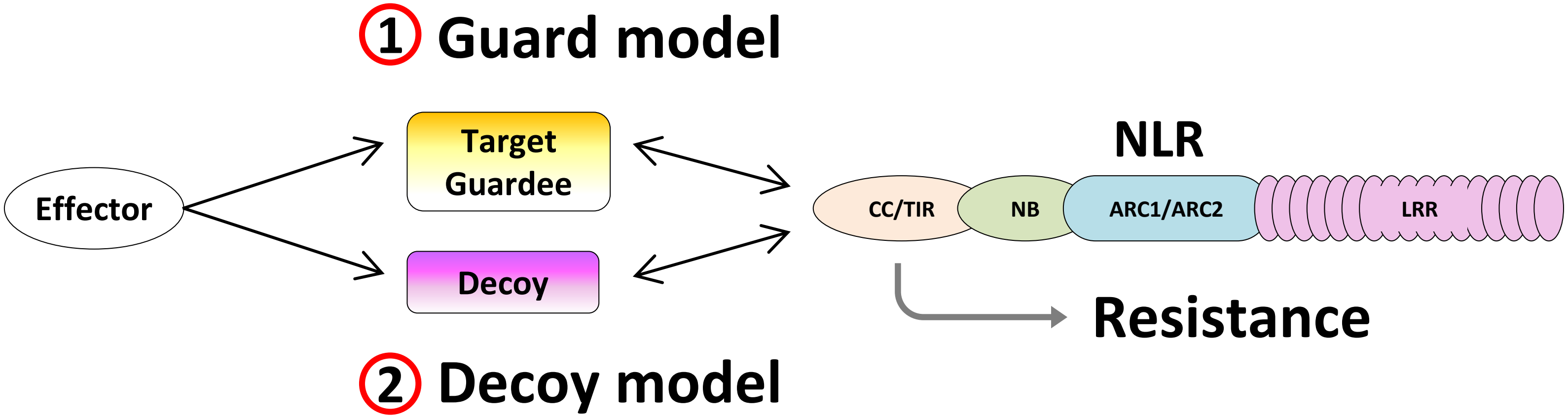
Sukarta et al. *Sem. Cell & Dev. Biol.* 2016

As for PRRs, plant genomes encode numerous NLRs



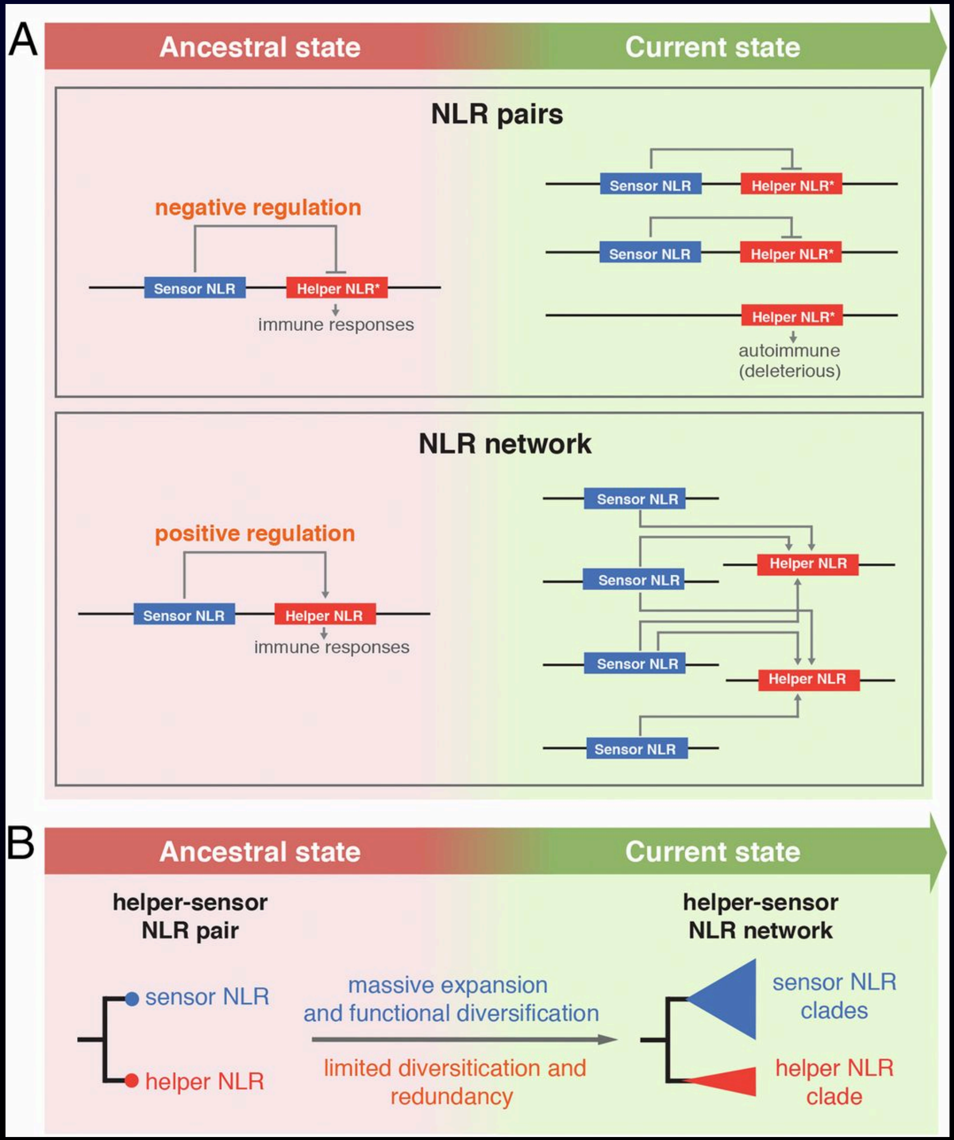
Jones et al., *Science*, 2016

Different modes of effector recognition by plant NLRs



Modified from: Cesari et al., *Front. Plant Sci.,* 2014; Wu et al., *Front. Plant Sci.,* 2015; Sarris et al., *BMC Biology* 2016

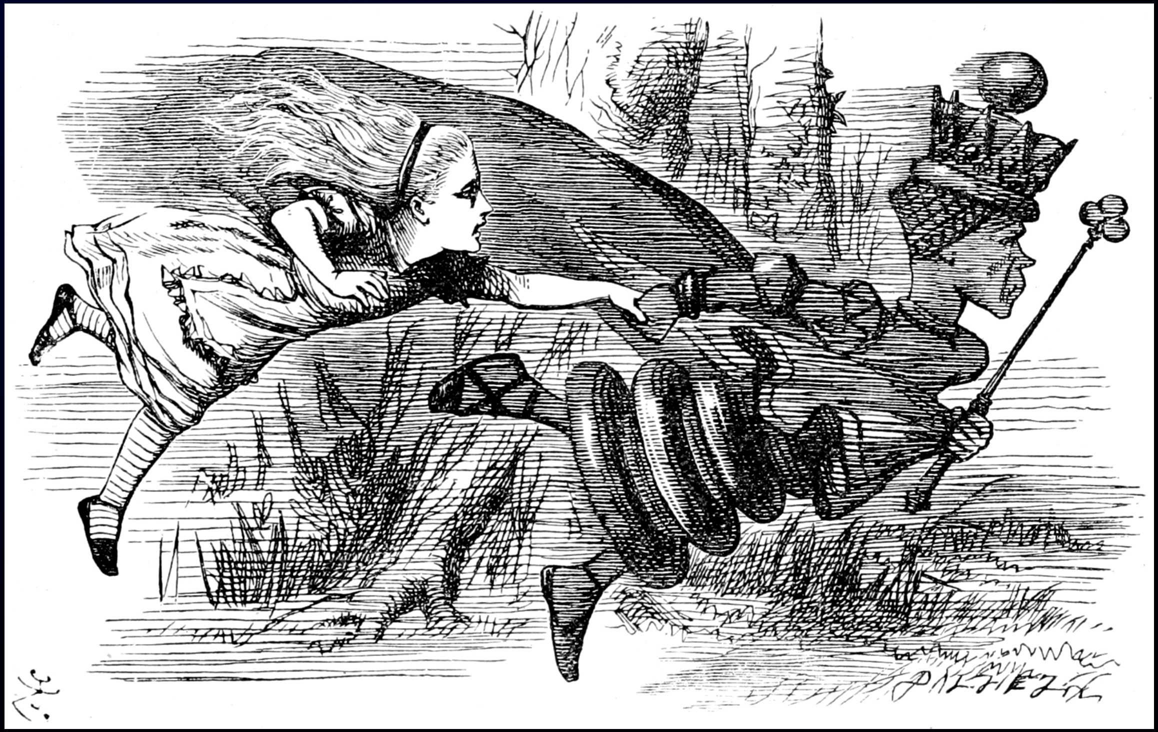
Sensing NLRs and helper NLRs



Wu et al., *PNAS*, 2017

Arms race during plant-pathogen interactions

Each study model represents a current snapshot of the constant evolutionary arms race between a given host and its pathogens

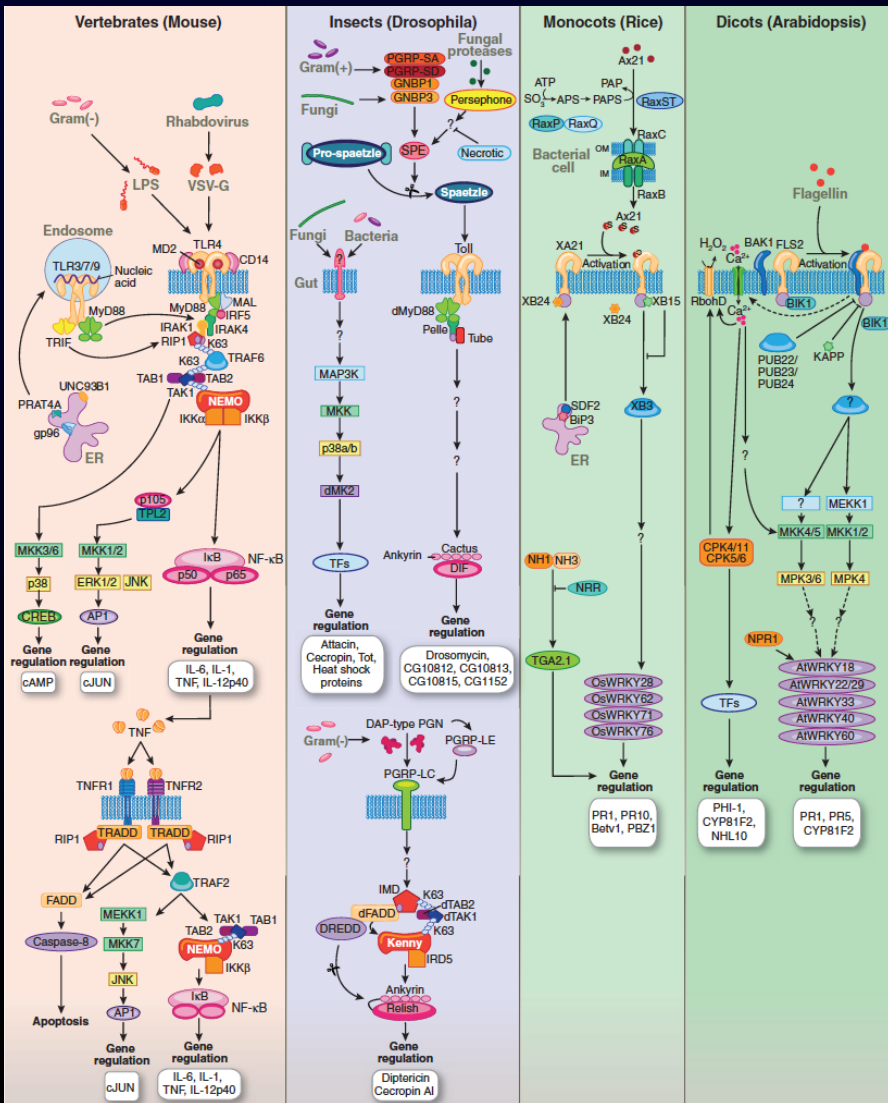


Common mechanisms in innate immunity: convergent evolution

Plant and Animal Sensors of Conserved Microbial Signatures

Pamela C. Ronald[1,2,3]* and Bruce Beutler[4]

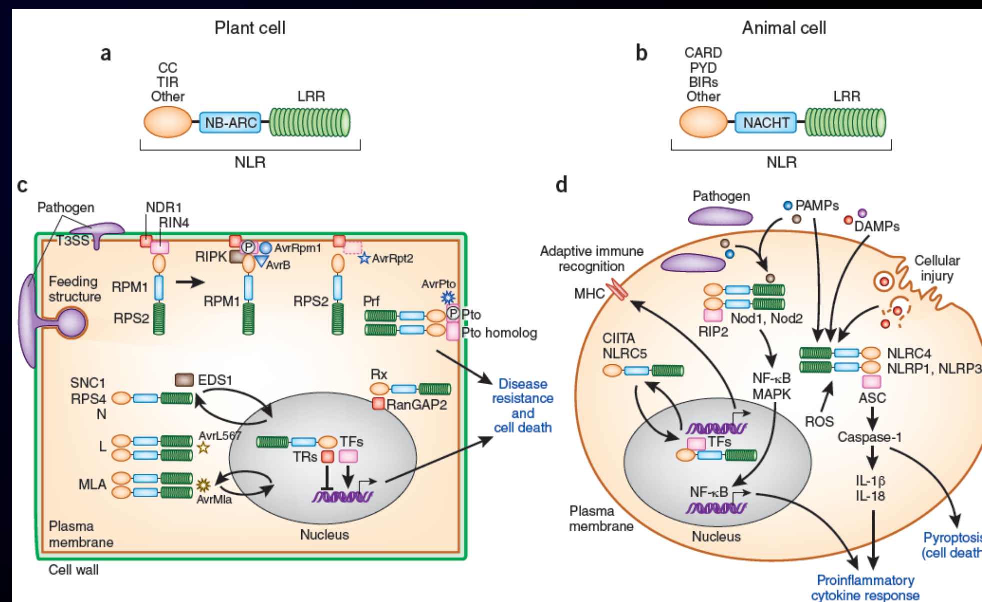
SCIENCE VOL 330 19 NOVEMBER 2010



VOLUME 12 NUMBER 9 SEPTEMBER 2011 NATURE IMMUNOLOGY

NLR functions in plant and animal immune systems: so far and yet so close

Takaki Maekawa[1], Thomas A Kufer[2] & Paul Schulze-Lefert[1]



Plant-pathogen interactions: a bit of jargon

Basal resistance

Level of resistance which remains when a virulent pathogen infects a susceptible plant (compatible interaction):

PTI - ETS (+ weak ETI)

Basal resistance cannot protect the plant against pathogens, but restricts its level of virulence.

Non-host resistance

Resistance which occurs when a normally virulent pathogen for a particular host is not able to infect and grow in a different.

Complex biological phenomenon which involves:

- Ecological factors
- Constitutive physical and biochemical barriers
- Inducible defence reactions: PTI, ETI

Importance of each components depends on the considered interaction.

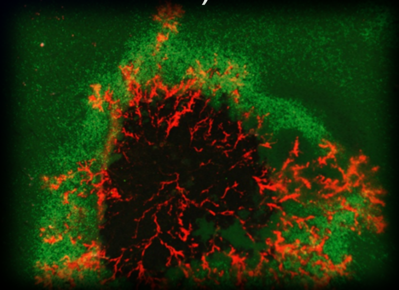
Plant-pathogen interactions: a bit of jargon

Biotrophic pathogens

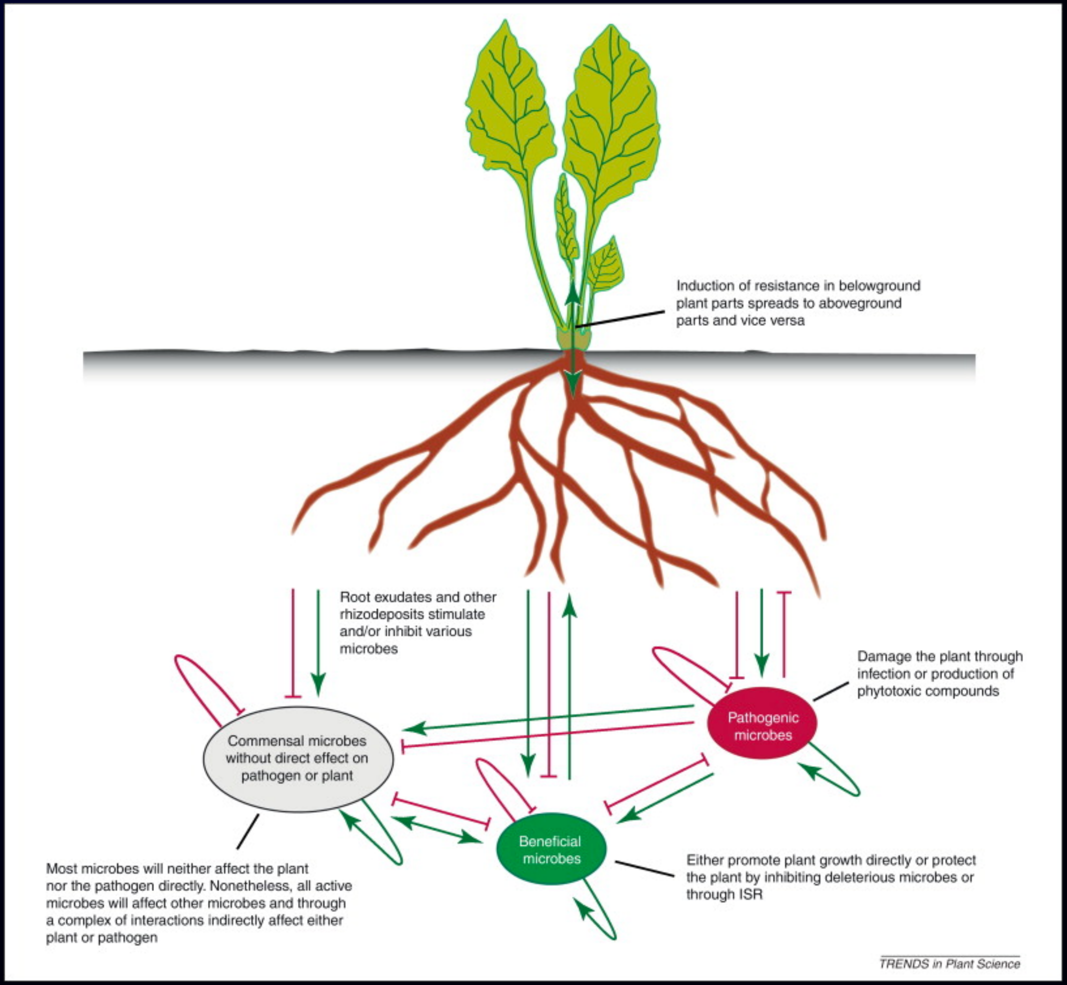
- Keep the host alive and reprogram the host physiology to its benefit.
- Resistance is often controlled by the immune hormone salicylic acid (SA).
- Hemibiotrophs: would still kill host cells late during infection (*e.g. Pseudomonas syringae*)
- Obligate biotrophs: cannot be cultivated without a leaving host plant (*e.g.* rusts; downy mildew)

Necrotrophic pathogens

- Kill host cellas as a way of life (*e.g.* to get nutrients).
- Resistance is often controlled by the immune hormones jasmonic acid (JA) and ethylene (ET).
- Many necrotrophs start with an early biotrophic phase (e.g. *Phytophthora infestans*).
- Many necrotrophs use non-specific toxins to kill host cells.
- ETI actually contributes here to virulence!



Symbionts and commensals: a disclaimer



Berendsen et al., *Trends Plant Sci.,* 2012

Relevant recent reviews:

Müller et al., *Annu. Rev. Genet.* 2016
Zipfel & Oldroyd, *Nature* 2017
Hacquard et al., *Annu. Rev. Phytopathol.* 2017
Cao et al., *Annu. Rev. Plant Biol.* 2017