MOLECULAR AND CELLULAR BASIS OF HOST RESPONSES TO THE PATHOGEN

PLANT DEFENSE RESPONSE

- NATURAL (AVOIDANCE)
  - ❖ **PREFORMED BARRIERS:**
    - ➢ **Structural**
    - ➢ **Biochemical**
  - ❖ NON-HOST RESISTANCE
- ACQUIRED
  - LOCAL
    - HYPERSENSITIVE RESPONSE (HR)
      - ➢ NO, ROS, ETHYLENE, Ca^{++} etc.
  - SYSTEMIC
    - SYSTEMIC ACQUIRED RESISTANCE (SAR)
      - ➢ SA
    - INDUCED SYSTEMIC RESISTANCE (ISR)
      - ➢ JA, ETHYLENE

Two Forms of Innate Immunity in Plants

- In classic textbooks, these two forms have been called **basal or horizontal disease resistance** and **resistance (R) gene-based or vertical disease resistance**; these two forms are now defined as **PAMP-triggered immunity (PTI)** and **effector-triggered immunity (ETI)** in a new terminology.
- **PTI (formerly called basal or horizontal resistance)** is based on the **PRR-mediated recognition of MAMPs and DAMPs**, the so-called general elicitors.
- **ETI (formerly called *R-gene-based or vertical* resistance)** is based on the **highly specific, direct or indirect interaction of pathogen effectors and the products of plant *R genes*** according to the gene-for-gene theory.
- This recognition event generally leads to a vigorous type of defence reaction called the hypersensitive response, characterized by **rapid apoptotic cell death and local necrosis**.

Plant defense system

- **Non host resistance**
- **Basal resistance/ Horizontal resistance (PTI)-** One key concept surrounding basal immune systems is that plant recognize certain **broadly conserved molecules associated with a wide range of pathogens**.
- **Vertical resistance (ETI)-** Pathogen-suppressed PTI response is backed up by a second layer of receptors called resistance (R) receptors that monitor the presence of pathogen effector proteins in what is called the effector-triggered immunity (ETI) of plants.
- **Systemic resistance (SAR and ISR)**

Non host defense : most common form of plant defense in nature

- **Resistance observed when all members of a plant species exhibit resistance to all members of a given pathogen species.**
- **Multigenic and multilayered; inactivation of anyone component may not be sufficient to render a plant susceptible.**
- **Variety of mechanism:**

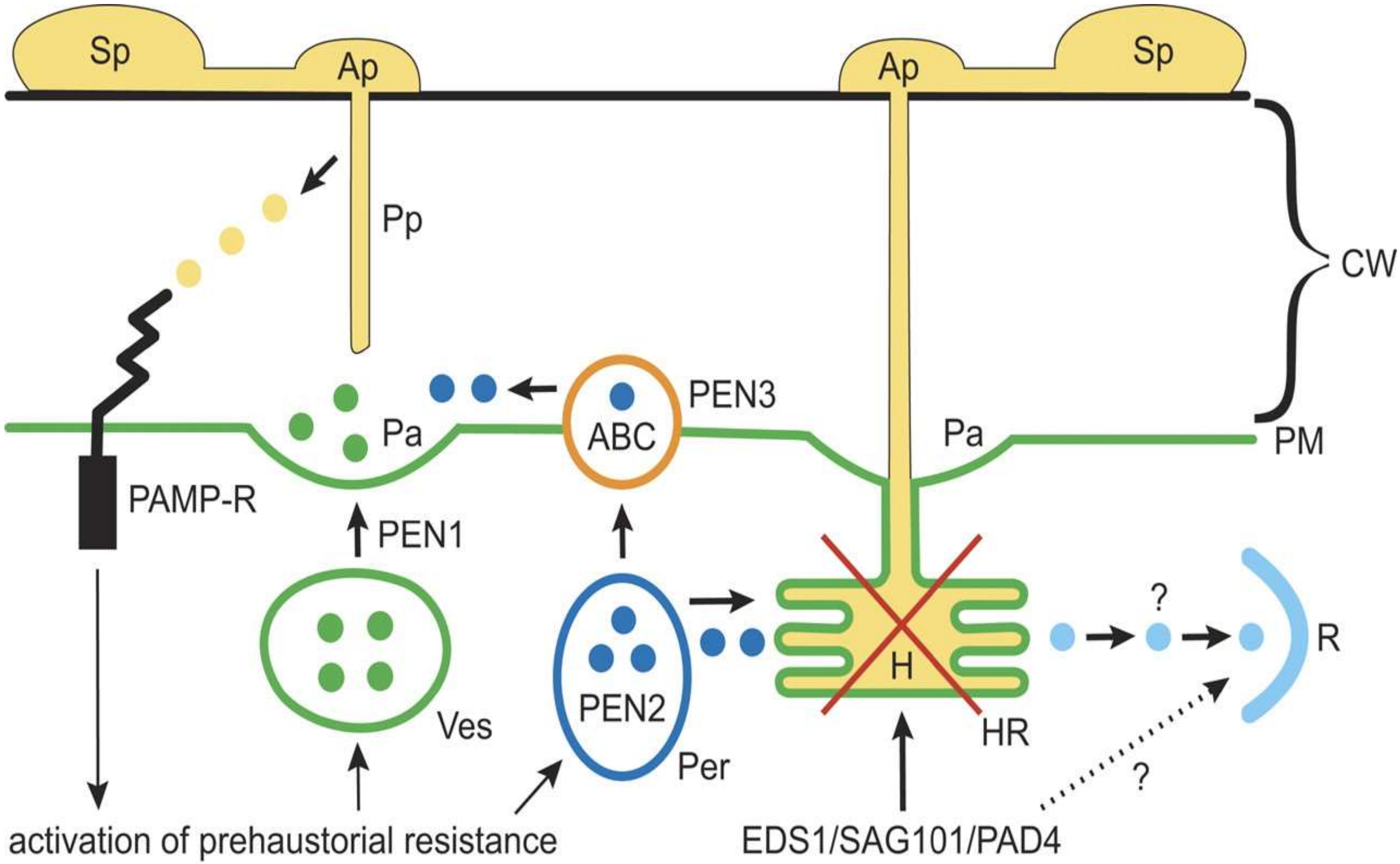
1. **Production of pre-formed toxins/barriers**
2. **Or lack of essential metabolites or signaling molecules required by the pathogen**

- **SUM OF PRE AND POST HAUSTORIAL RESISTANCE COMPONENTS**

The Molecular Basis of Non Host Resistance

- A dynamic process involving **organelle movement, secretion processes, membrane changes, and accumulation of three PEN proteins at the infection site**
- Pre haustorial resistance controlled by three genes, PEN1, PEN2, and PEN3, involved in biosynthetic and secretion processes
- **PEN1** – **vesicle based pathway, encodes syntaxin.**
- **PEN2** – **peroxisome based pathway, encodes glycosyl hydrolases**
- **PEN3** – **membrane localized ABC transporter protein PDR8.**
- Post haustorial resistance is dependent on genes – **PAD4, EDS1 and SAG101.**
- A cytoskeletal based mechanism that involves vesicle movement and exocytosis appears to be the basis of non host resistance.

Schematic Representation of *Arabidopsis* Nonhost Resistance to *Bgh*



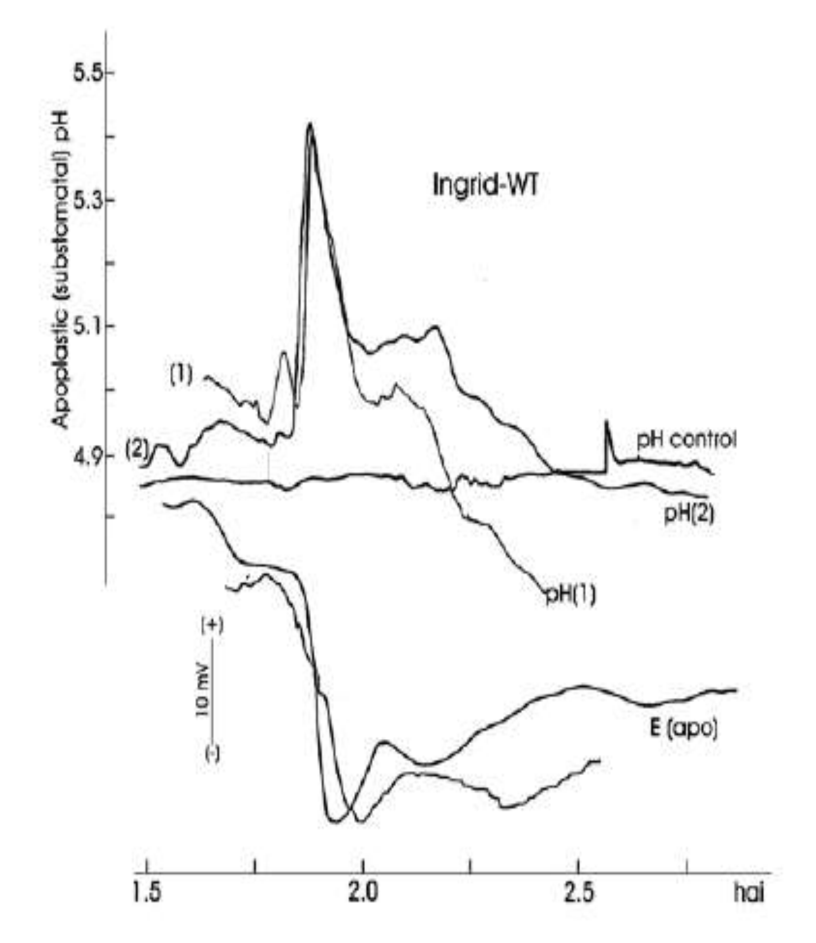
Ellis,J. 2006

Plant Basal Defense Responses

- **Race non-specific host resistance that is activated in susceptible plants and limits the severity of disease.**
- **Based on the recognition of PAMPs**
- **Induce MAP kinase signaling cascades and transcriptional activation of defense related genes. Oxidative burst and changes in apoplastic pH.**
- **Associated with rapid deposition of β-1,3, glucan callose, a physical barrier at the site of infection**
- **Callose is used as a molecular marker of basal defenses**
- **Cell wall associated defense is important on basal defense.**

Apoplastic pH signaling during *Barley - Blumeria graminis hordei* interaction

- Apoplastic alkalinization and acidification – potential signaling events in apoplast and cytosol.
- Apoplastic alkalinization – related to ROS production and resistance
- Low pH- maximize activity of cell wall degrading enzymes and have role in fungus induced host cell wall loosening (susceptibility).



Felle *et al.*, 2004

Plant antimicrobial compounds

- Low molecular weight compounds, such as induced phytoalexins, constitutive phytoanticipins and antimicrobial proteins.
- **Phytoalexins** – inhibit fungal development and crucial for penetration resistance together with induced mechanical strengthening of the cell wall.
- **PR proteins** – secreted into the cell wall during basal resistance responses; Affect fungal cell wall or membrane integrity such as **chitinases, glucanases, thionins, osmotins, proteases and defensins.**

Plant Cell Wall Strengthening

- Cell wall penetrating fungi frequently trigger the formation of heterogenous deposits between the plant plasma membrane and cell wall known as Cell Wall Apposition or Papillae.
- Presence of hydrogen peroxide in cell wall appositions is a biochemical marker.

Mechanism

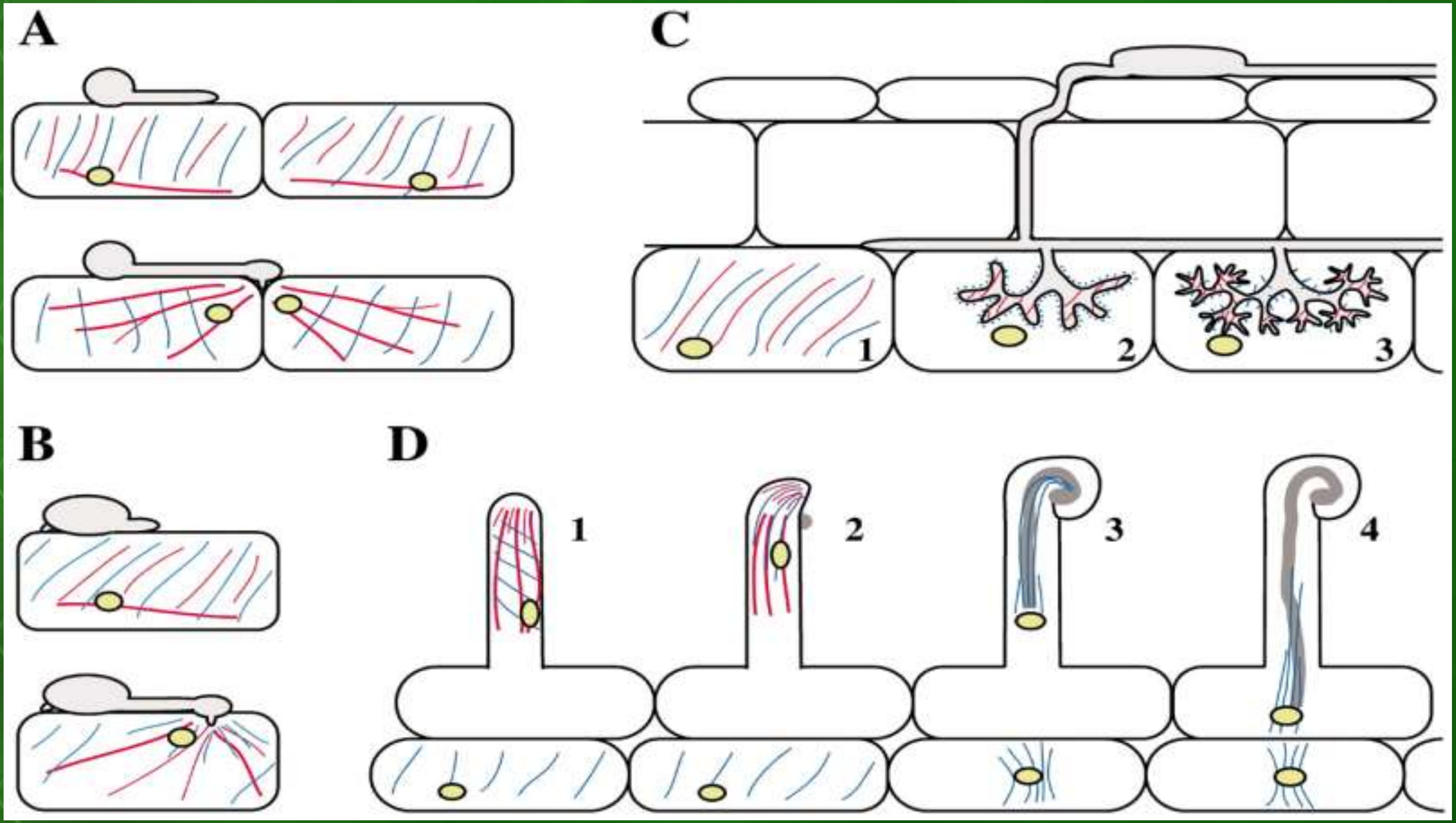
- Either a physical or a chemical barrier to fungal ingress
- Silica, an impermeable compound and provide a barrier to the exchange of nutrients or molecules in plant pathogen dialogue as well as physical barrier to penetration.
- Contains potentially toxic molecules.

- **Lignification – makes cell wall resistant to mechanical pressure applied during penetration by fungal appresoria.**
- **Cell walls become water resistant and less accessible to cell wall degrading enzymes.**
- **Callose – main structural component of many papillae**
- **In *Arabidopsis*, POWDERY MILDEW RESISTANCE 4 (PMR4) and GLUCAN SYNTHASE 5 (GLS5) genes both codes for the same callose synthase involved in wounding and papillary response.**

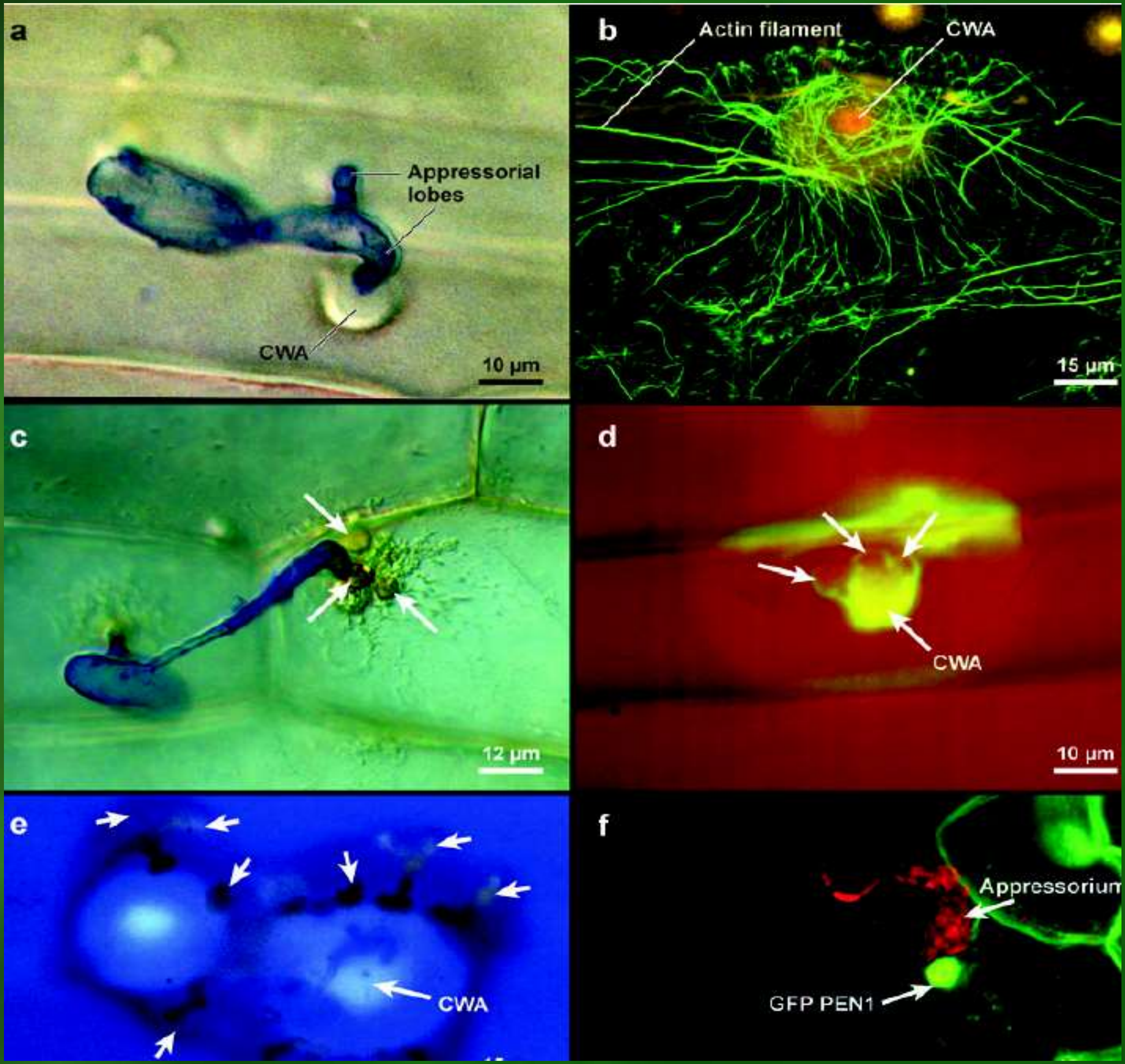
Cytoplasmic Rearrangements

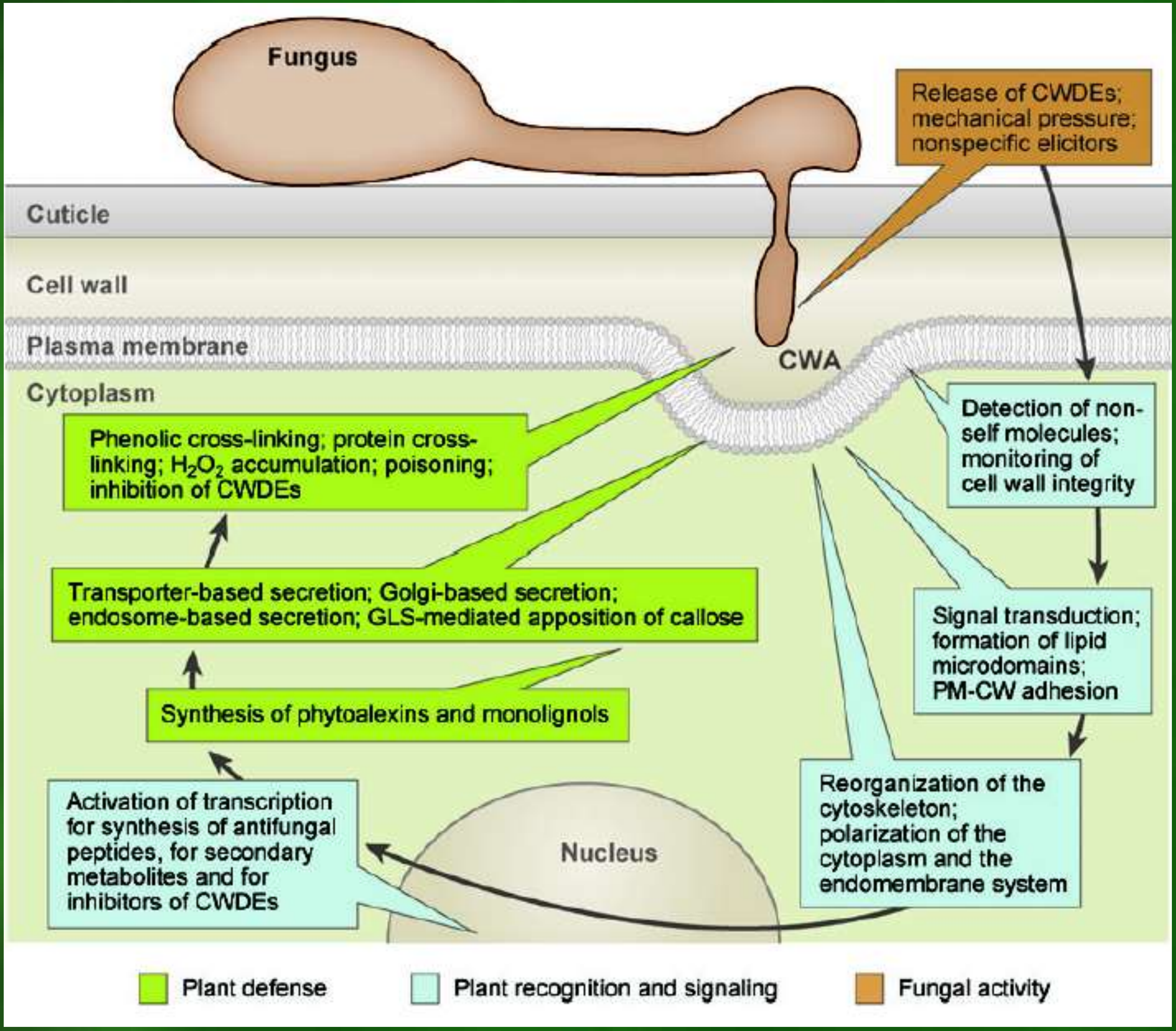
- **Cytoplasmic reorganization depends on filamentous actin and on microtubules.**
- **Direction of polarization and amount of cell wall associated defenses may thereby depend on local elicitor perception or mechanical pressure.**
- **During fungal attack the cytoskeleton and the endomembrane system are dramatically rearranged and polarized.**
- **The plasma membrane microdomains form at the penetration site and contains defense related proteins such as AtPEN1, AtPEN2, AtPEN3, HvROR2, and HvMLO.**
- **ER and Golgi bodies accumulates at penetration site.**
- **HvROR1, HvROR2, and HvMLO – formation of large vesicle-like bodies at CWAs in barley attacked by *Bgh***

Diagrammatic Representation Of The Organization Of The Plant Cytoskeleton During Different Plant - Microbe Interactions



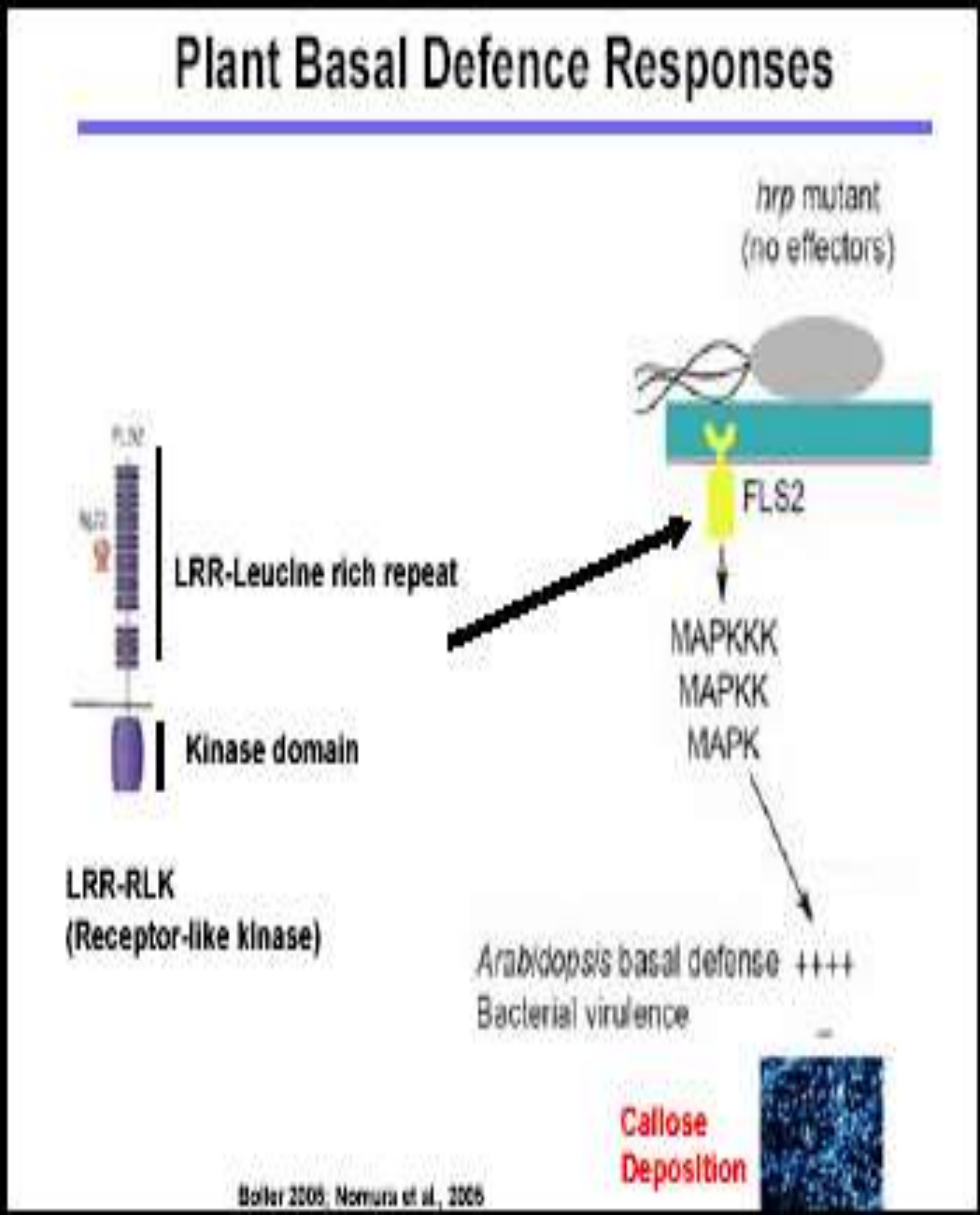
Takemoto, D. *et al*., 2004


a
Appressorial lobes
CWA
10 µm
b
Actin filament
CWA
15 µm
c
12 µm
d
CWA
10 µm
e
CWA
f
Appressorium
GFP PEN1


Fungus
Release of CWDEs; mechanical pressure; nonspecific elicitors
Cuticle
Cell wall
Plasma membrane
CWA
Cytoplasm
Phenolic cross-linking; protein cross-linking; H_2O_2 accumulation; poisoning; inhibition of CWDEs
Detection of non-self molecules; monitoring of cell wall integrity
Transporter-based secretion; Golgi-based secretion; endosome-based secretion; GLS-mediated apposition of callose
Signal transduction; formation of lipid microdomains; PM-CW adhesion
Synthesis of phytoalexins and monolignols
Activation of transcription for synthesis of antifungal peptides, for secondary metabolites and for inhibitors of CWDEs
Nucleus
Reorganization of the cytoskeleton; polarization of the cytoplasm and the endomembrane system
Plant defense
Plant recognition and signaling
Fungal activity

Flagellin perception : Perception of PAMP and PTI

- **Flagellin** - bacterial flagella protein ; 22 amino acids
- **FLS2** – host plasma membrane receptor-like kinase
- Recognition of flagellin by FLS2 leads to the activation of MAP (mitogen–activated protein) kinase cascade and phosphorylation of WRKY transcription factor .
- Discovery of **BAK 1.**



Recently discovered key components in PAMP signaling

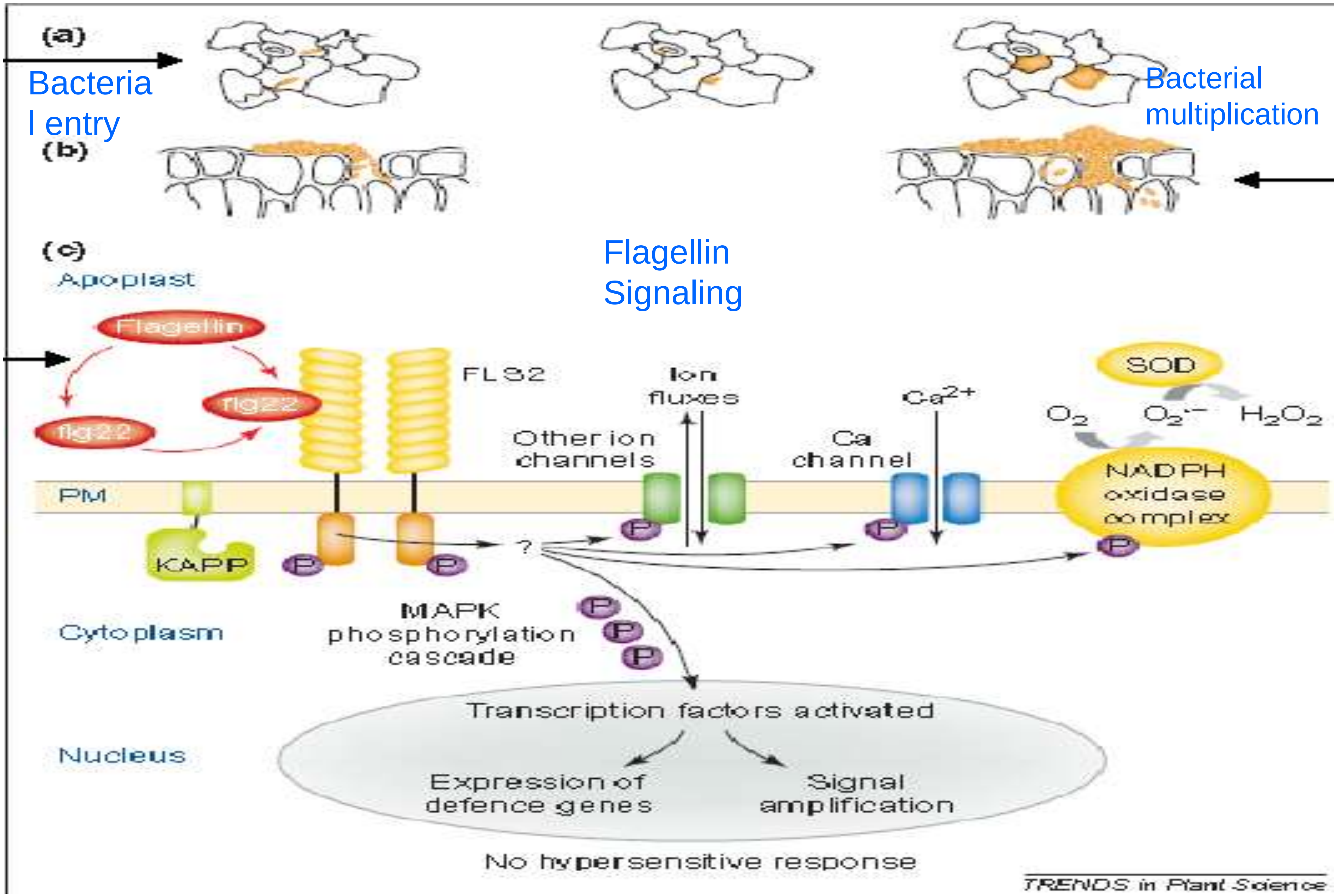
- **Brassinosteroid insensitive 1 (BR11)-associated receptor kinase 1 (BAK1)** mediating BR signaling in Arabidopsis and tobacco.
- **BAK1** – co-immunoprecipitation partner for Arabidopsis FLS2.
- Once interacting with flg22, the FLS2-BAK1 complex initiates a set of path-related responses such as MAPK activity and the oxidative burst.

COR and PTI associated guard cell responses- the closure of stomata in response to bacteria and PAMPS is one part of the PTI.

This PTI response required synthesis of nitric oxide, Salicylic acid or abscisic acid (ABA).

MAPK signaling and ABA signaling are two different signaling pathways for stomata closure.

Flagellin Signaling In Plants

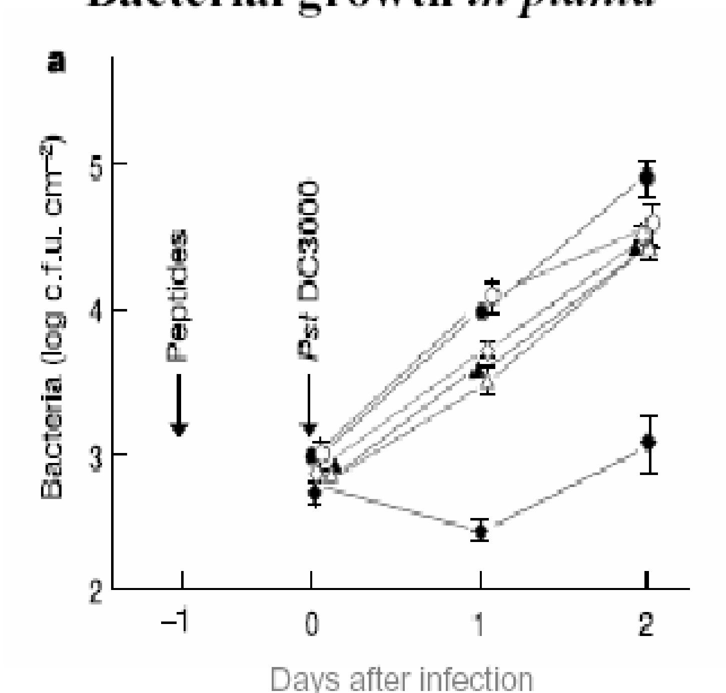


Bacterial disease resistance in *Arabidopsis* through flagellin perception

NATURE | VOL 428 | 15 APRIL 2004

Cyril Zipfel[1]*, Silke Robatzek[1]*, Lionel Navarro[2], Edward J. Oakeley[1], Jonathan D. G. Jones[2], Georg Felix[1]* & Thomas Boller[1]*

Bacterial growth *in planta*



Elicitor active flagellin is important for basal defense

Flagellin & defense signaling

Table 1 flg22-induced resistance in plants affected in salicylic acid, jasmonic acid and ethylene signalling

	Bacterial count (log c.f.u. cm^{-2})	
Line	flg22^{Atum}	flg22
Col-0	4.6 ± 0.2	3.4 ± 0.2
NahG	5.9 ± 0.01	4.7 ± 0.1
etr1-3	4.4 ± 0.3	2.7 ± 0.2
ein2-1	3.5 ± 0.25	2.7 ± 0.3
jar1-1	4.6 ± 0.15	3.5 ± 0.2
pad2-1	5.0 ± 0.1	3.9 ± 0.3
pad4-1	5.5 ± 0.2	3.6 ± 0.2
Ler-0	5.1 ± 0.1	3.5 ± 0.1
fls2-17	4.8 ± 0.25	4.9 ± 0.1
eds1-2	5.6 ± 0.05	3.7 ± 0.2
sgt1b-3	5.0 ± 0.2	3.6 ± 0.05
rar1-13	5.2 ± 0.2	3.5 ± 0.2
No-0	5.0 ± 0.2	2.6 ± 0.25
npr1-5	4.5 ± 0.15	2.2 ± 0.05

Flagellin induced resistance is independent of SA, JA & ethylene signaling

ETI : Cell Death at the Center of Immune Responses

- The first observations of HR in 1902 in the *wheat-Puccinia glumarum* pathosystem, and the term ‘hypersensitiveness’ was coined in 1915 by E.C.Stakman (*Pgt*-wheat pathosystem).
- Morphologically, HR is a specific and unique type of cell death. Its hallmarks: **cytoplasmic shrinkage, chromatin condensation, mitochondrial swelling**, **vacuolization and chloroplast disruption** (plant specific characters) during the final stages.
- **The chloroplast has a central role in defense responses and HR in plants.**

- I- Source of defense signaling molecules such as reactive oxygen species (ROS), reactive nitrogen oxide intermediates (NOI) and the defense hormones salicylic acid (SA) and jasmonic acid (JA).
- II- In many cases, light is required for HR development.
- III- Several effectors have chloroplast localization signals, and in some cases they have been shown to suppress immunity.
- **In plants, the molecular events that lead to HR during ETI are partly overlapping with those associated with PTI, including accumulation of SA, ROS and NOI, activation of MAPK cascades, changes in intracellular calcium levels, transcriptional reprogramming and synthesis of antimicrobial compounds.**

Hypersensitive Response/ Programmed Cell Death

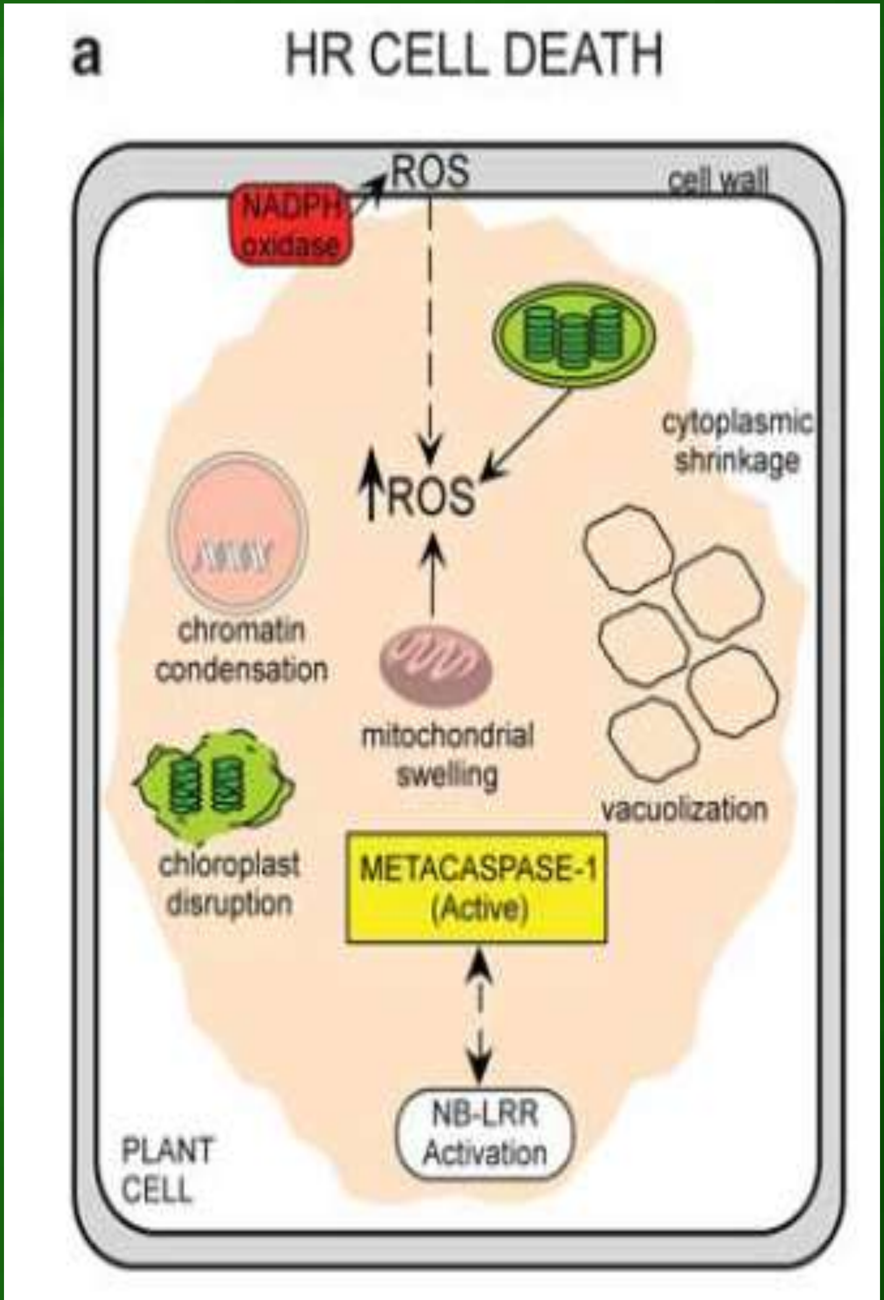
"Hypersensitive response is rapid localised cell death which results in the formation of necrotic lesions around infection sites."

- A last line of defense to pathogens
- Resultant of R-Avr incompatible interaction
- Directly kill and damage pathogens
- Restrict pathogen spread

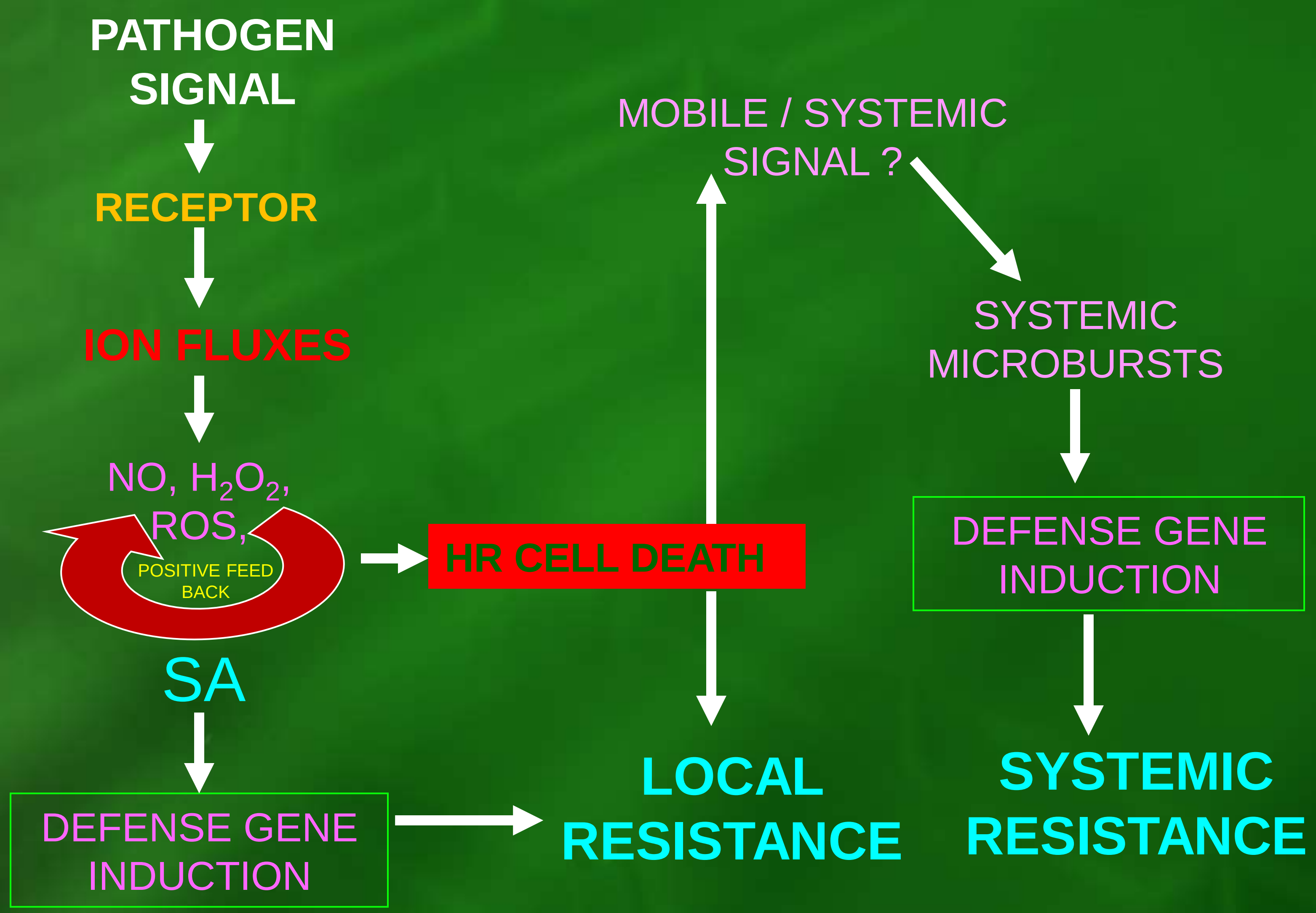
Characteristics of HR

- Burst of oxygen reactive species, H_2O_2, NO, Ca^{++} and ion fluxes
- Accumulation of Salicylic Acid (SA)
- Antimicrobial phytoalexins
- Strengthen cell walls, and triggers apoptosis

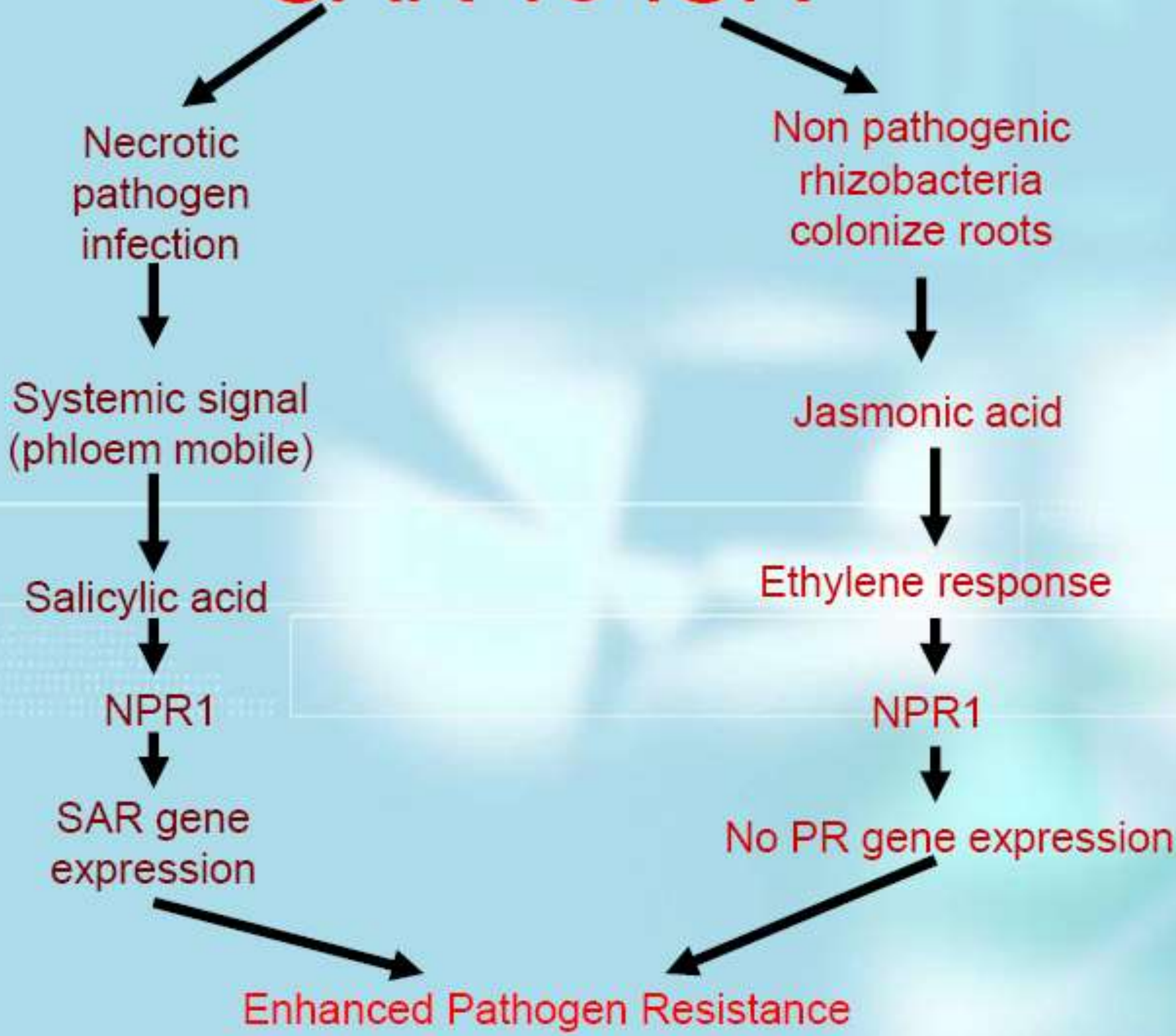
- HR, which is part of the effector-triggered immunity (ETI), starts with an efflux of hydroxide and potassium and an influx of calcium and hydrogen ions into the cell. Cells involved in HR produce reactive oxygen species (ROS) which damage the cell membrane. The result is the formation of lesions which prevent the spread of infection.



HR Initiate Systemic Resistance



SAR vs ISR

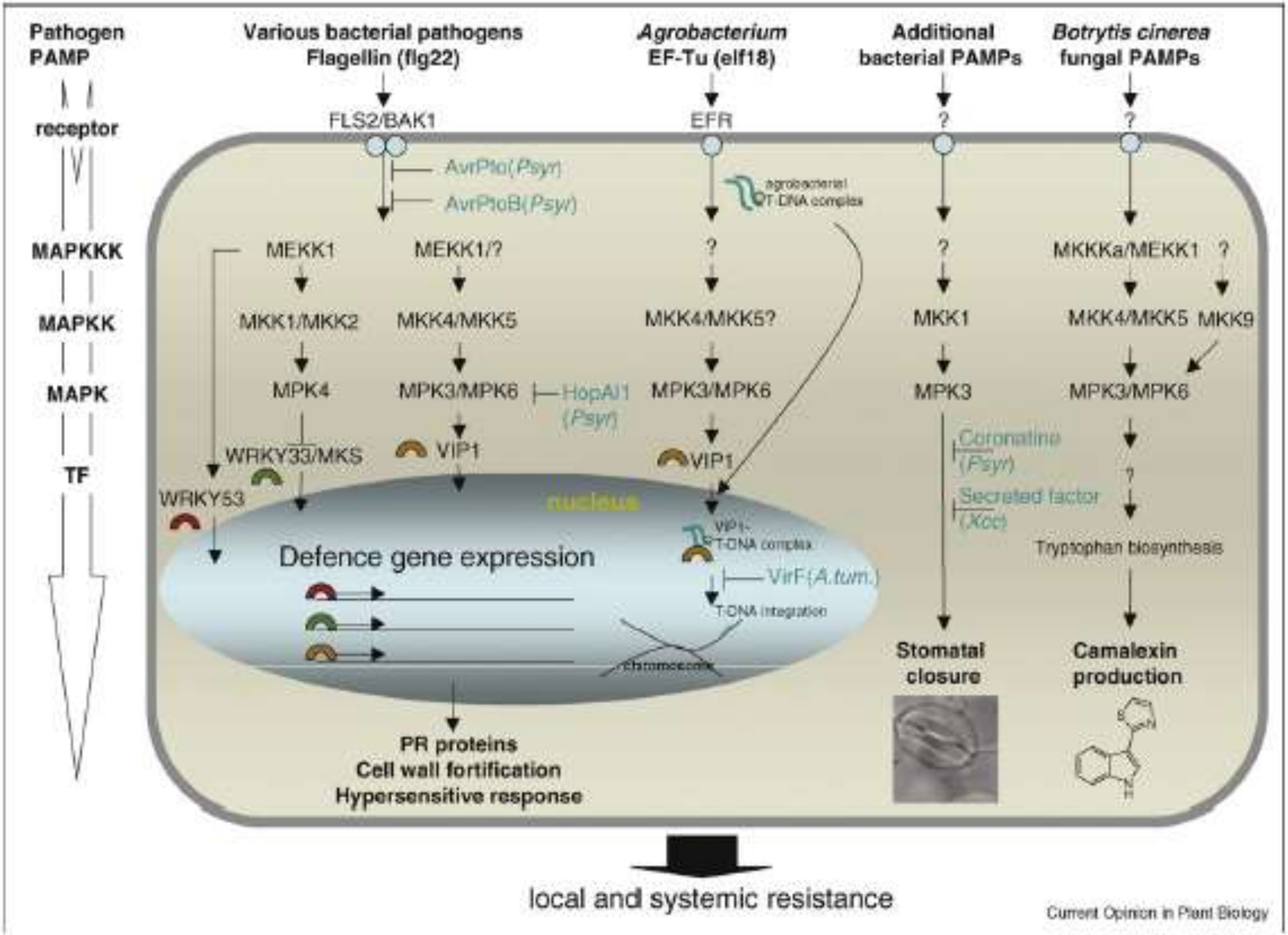

Necrotic pathogen infection
Systemic signal (phloem mobile)
Salicylic acid
NPR1
SAR gene expression
Non pathogenic rhizobacteria colonize roots
Jasmonic acid
Ethylene response
NPR1
No PR gene expression
Enhanced Pathogen Resistance

SIGNALLING IN PLANT DISEASE RESISTANCE MECHANISMS

MAPKinase Cascade

- MAPK cascades are conserved signaling modules found in all eukaryotic cells, which transduce and amplify extracellular and intracellular stimuli into acidic range of overlapping or specific intracellular responses in eukaryotic cells.
- Mitogen associated protein kinases are serine/threonine specific protein kinases that responds to extracellular stimuli (reactive oxygen species, osmotic stress, heat shock,UV, drought and pathogen attacketc) and regulates various cellular activities , such as gene expression, mitosis, differentiation, proliferation, and cell survival or apoptosis.
- MAPKinases are activated within the protein kinase cascades known as MAPK cascades.

- Each one consists of 3 enzymes: MAPKinase, MAPKinase kinase (MKK, MEK/MAP2K), MAPKinase kinase kinase (MKKK, MEKK or MAP3K) that are activated in series.
- A MAP3K that is activated by extracellular stimuli phosphorylates a MAP2K on its serine and threonine reisdues, and this MAP2K activates a MAPKinase through phosphorylation on its threonine and tyrosine residues (Tyr-185 and Thr-183 of ERK2).
- **The MAPkinase signaling cascades convey information to effectors, co-ordinate incoming information from other signaling pathways, amplify signals and allow a variety of response patterns.**
- They responds to different stimuli by phosphorylating cytoplasmic components and nuclear transcription factors depending on the cellular content.


Pathogen PAMP
receptor
MAPKKK
MAPKK
MAPK
TF
Various bacterial pathogens Flagellin (flg22)
Agrobacterium EF-Tu (elf18)
Additional bacterial PAMPs
Botrytis cinerea fungal PAMPs
FLS2/BAK1
EFR
?
AvrPto(Psyr)
AvrPtoB(Psyr)
agrobacterial T-DNA complex
MEKK1
MEKK1/?
MKKKa/MEKK1
MKK1/MKK2
MKK4/MKK5
MKK4/MKK5?
MKK1
MKK9
MPK4
MPK3/MPK6
MPK3
HopAI1 (Psyr)
WRKY33/MKS
VIP1
WRKY53
Coronatine (Psyr)
Secreted factor (Xcc)
nucleus
Defence gene expression
VIP1-T-DNA complex
VirF(A.tum.)
T-DNA integration
chromosome
Tryptophan biosynthesis
Stomatal closure
Camalexin production
PR proteins
Cell wall fortification
Hypersensitive response
local and systemic resistance
Current Opinion in Plant Biology

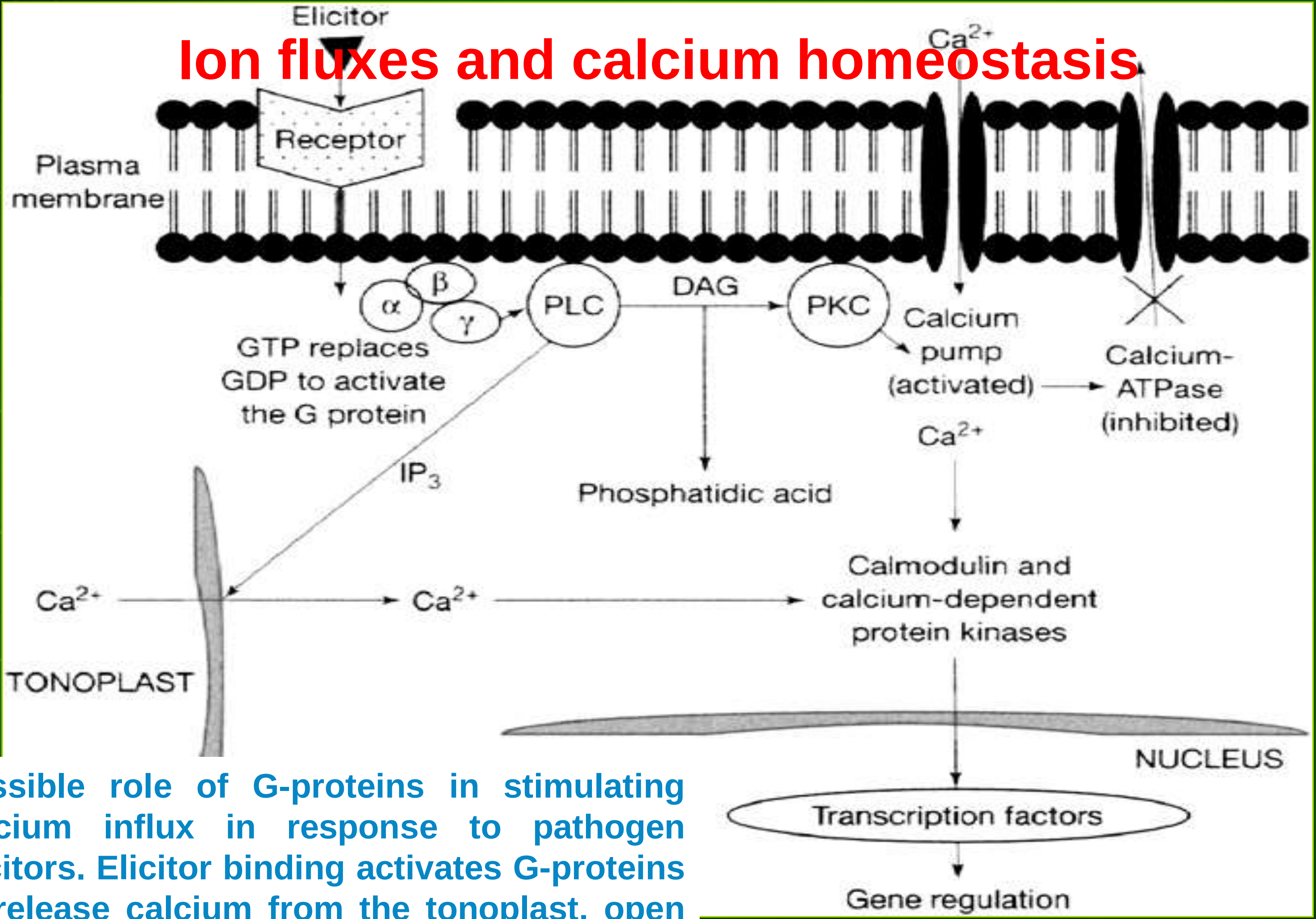
MPK3/MPK6 are necessary to induce defence responses

- MPK3 and MPK6 are closely related proteins that show a high level of functional redundancy. Both MAPKs are key regulators of a diverse set of processes including **abscission, stomatal development, signal various abiotic stresses and defence response to bacterial and fungal pathogens**.
- In plants, activated MAPKs are often transported to the nucleus where they phosphorylate specific transcription factors, **wound-induced protein kinase (WIPK), and salicylic acid-induced protein kinase (SIPK),** involved in the plant immune response. This response can lead to **programmed cell death.**
- Other MAPKs act as negative regulators of the immune responses. One model suggests that MAPK4 regulates JA signal transduction while blocking the MAPK that regulates the SA response in *Arabidopsis.*

•**MPK3/MPK6 are compulsory in camalexin biosynthesis**: Camalexin is required for resistance to *B. cinerea.*
•**MPK3 is required for stomatal immune responses.**

•**Negative regulation of defence responses by the MPK4 pathway :** A negative regulatory role of the MEKK1-MKK1/2-MPK4 module in SA and H_2O_2 production has been proposed.

Ion fluxes and calcium homeostasis



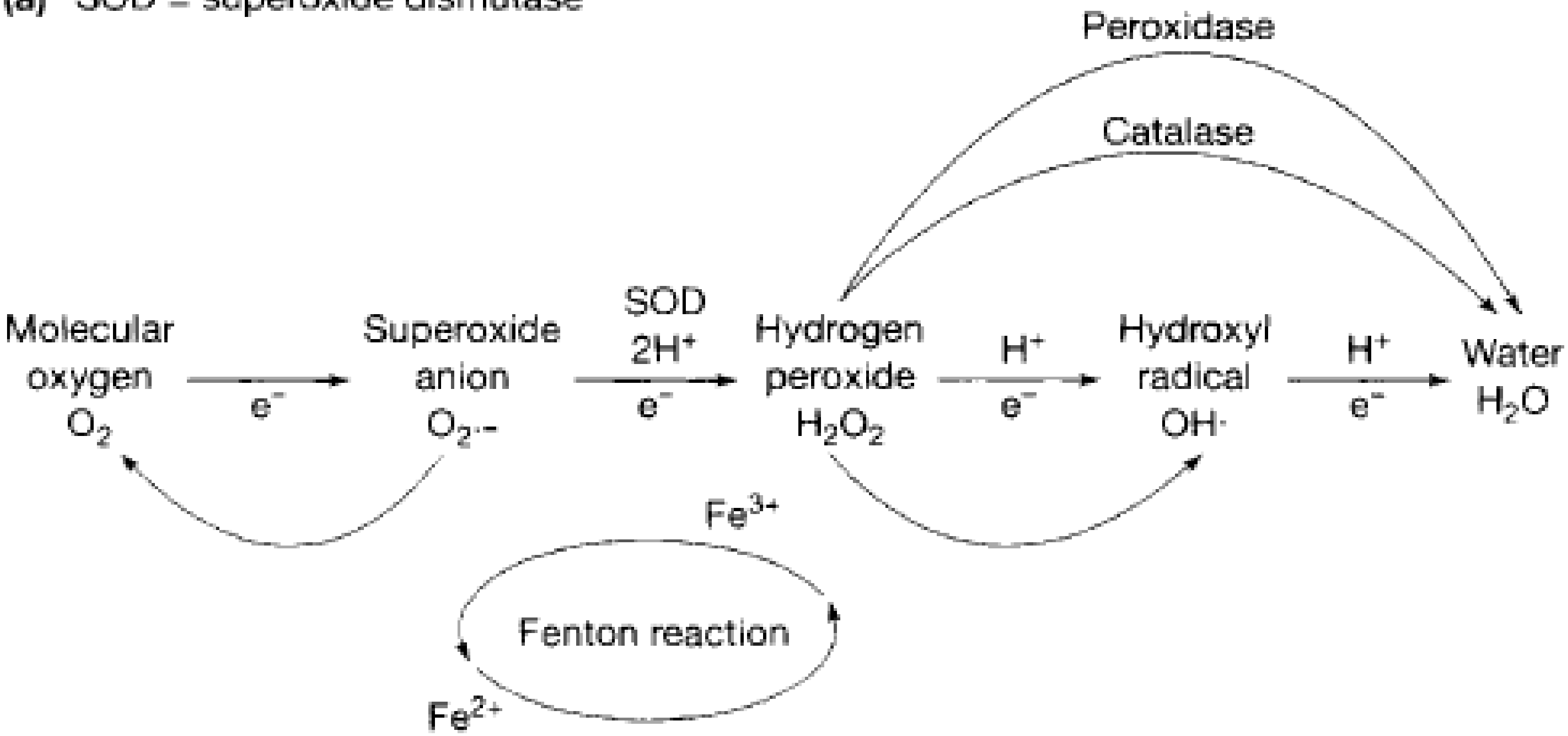
Possible role of G-proteins in stimulating calcium influx in response to pathogen elicitors. Elicitor binding activates G-proteins to release calcium from the tonoplast, open plasma membrane calcium channels, and inhibit the calcium-ATPase. The result is an influx of calcium and blockage of its efflux.

The oxidative burst

- **Response to elicitor perception and fungal cell wall penetration**
- **Reactive oxygen species : molecules like hydrogen peroxide, ions like hypochlorite ion, radicals like the hydroxyl radical.**
- **Regulation of apoplastic ROS production by pH, small monomeric GTPases, heteromeric GTPases, increased cytosolic Ca^{+2}, protein kinases, MAP kinases and extracellular ATP.**
- **Functions: in signaling and expression of defense gene, phytoalexin production, and both in HR and restriction of pathogen induced cell death**
- **Sources for ROS production : phagocyte respiratory burst oxidase homologous (RBOH), NADPH oxidases, peroxidases, amine oxidases, and oxalate oxidases.**
- **Defense against ROS : Super oxide dismutase and catalases**

General scheme for the production of reactive oxygen species by sequential reduction of molecular oxygen. (a) SOD=superoxide dismutase. (b) The Fenton reaction.

(a) SOD = superoxide dismutase

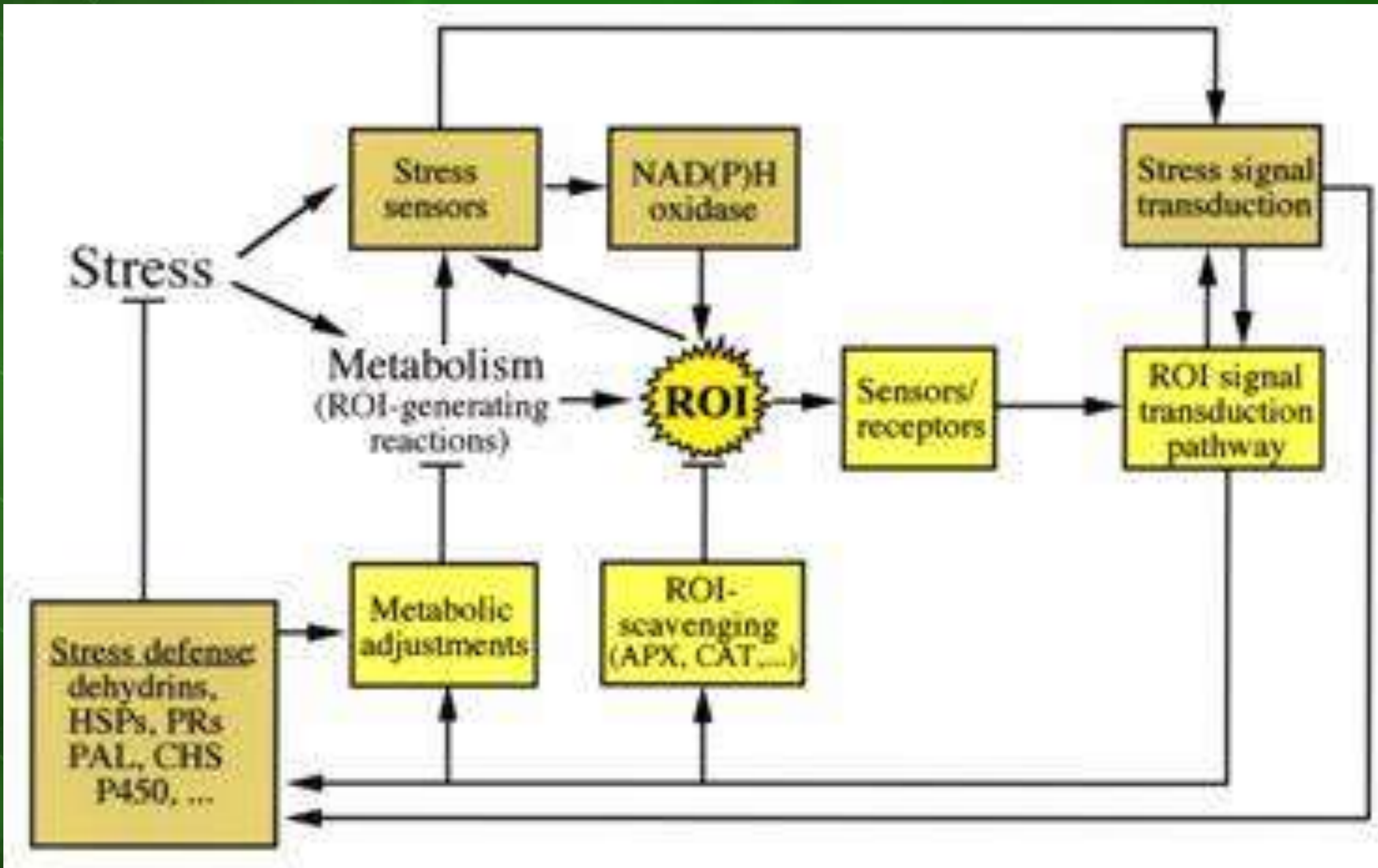


(b) The Fenton reaction

$$H_2O_2 + Fe^{2+} (Cu^+) \longrightarrow Fe^{3+} (Cu^{2+}) + OH\cdot + OH^-$$

$$O_2\cdot- + Fe^{3+} (Cu^{2+}) \longrightarrow O_2 + Fe^{2+} (Cu^+)$$

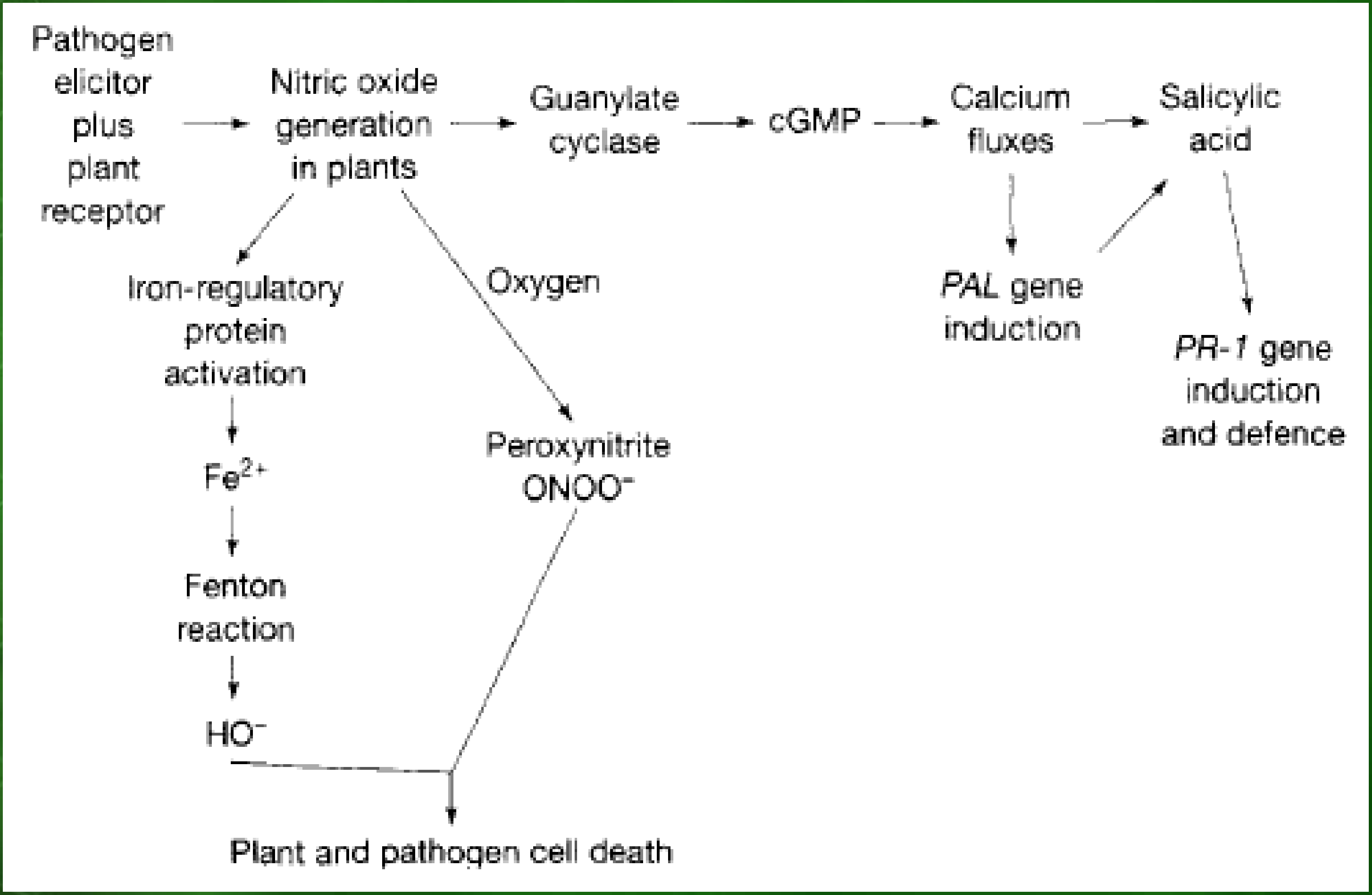
The ROS Cycle



Nitric oxide (NO)

- In plants, NO is known to be produced both non-enzymatically and through the action of NAD(P)H-dependent nitrate reductase.
- Evidence from the use of nitric oxide synthase (NOS) inhibitors, and antibodies raised against mammalian NOSs, has indicated that Ca2+-dependent NOS-like proteins are also present in plants.
- Moreover, synthesis of NO is induced in incompatible interactions in soybean and tobacco experiments, but not in compatible interactions, and the addition of NO donors or recombinant mammalian NOS to tobacco plants or cell suspensions induced expression of the *PR-1 and PAL genes.*

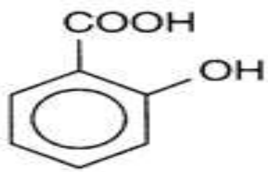
Proposed role for nitric oxide in defence responses in plants



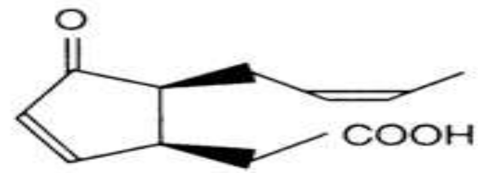
Low-molecular-weight signalling molecules

(a)

Salicylic acid



Jasmonic acid

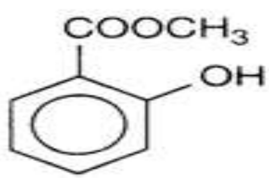


Systemin

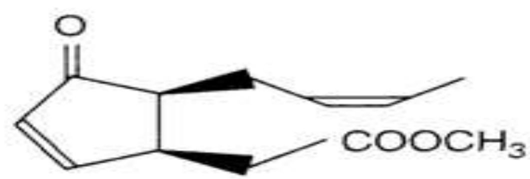
NH_2-Ala-Val-Gln-Ser-Lys-Pro-Pro-Ser-Lys-Arg-Asp-Pro-Pro-Lys-Met-Gln-Thr-Asp-COOH

(b)

Methyl salicylate



Methyl jasmonate

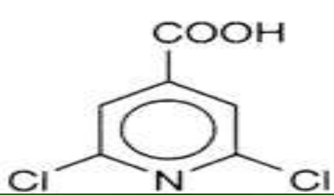


Ethylene

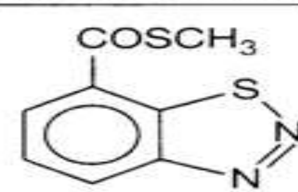
$H_2C = CH_2$

(c)

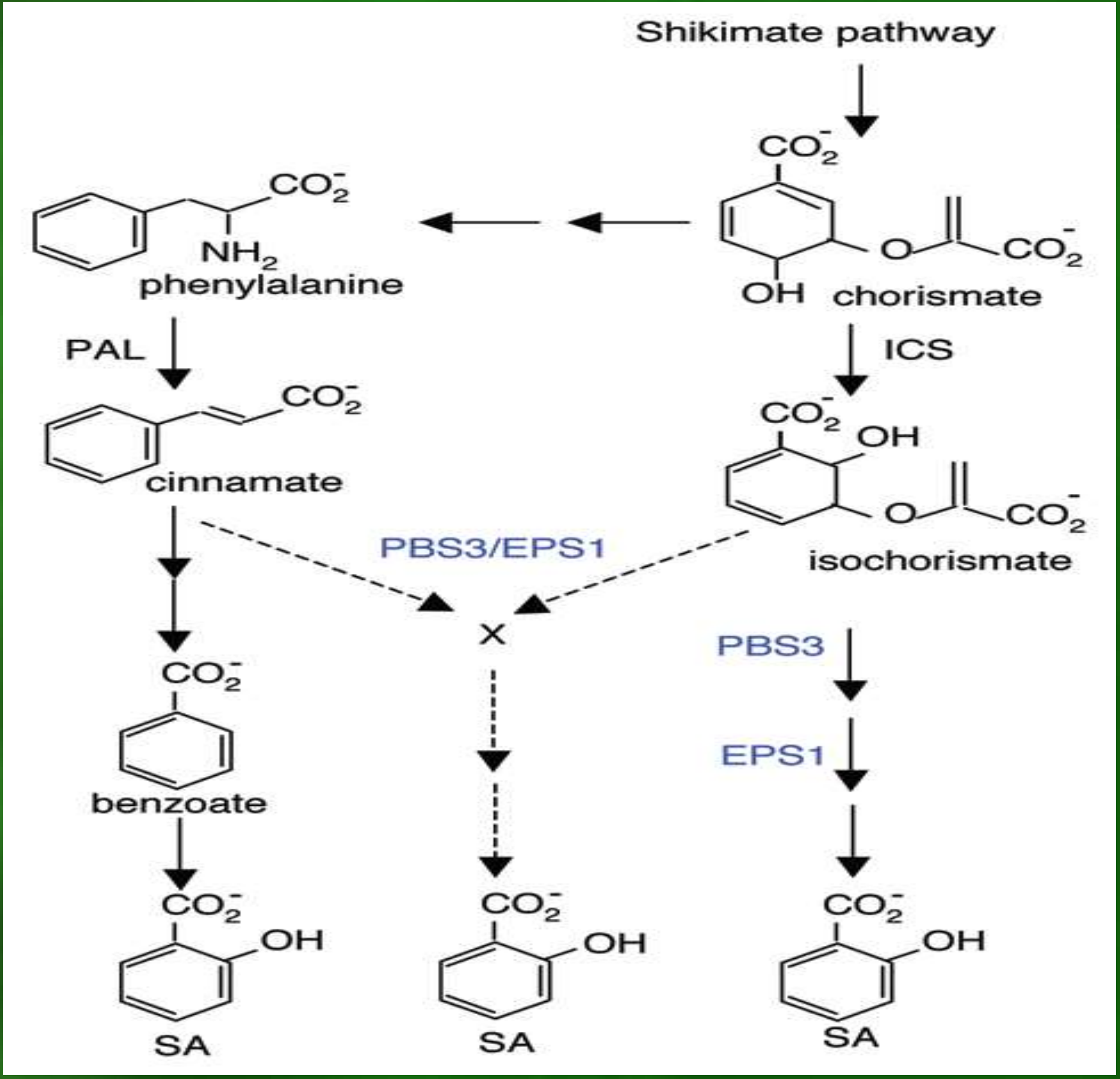
2,6 dichloroisonicotinic acid (INA)



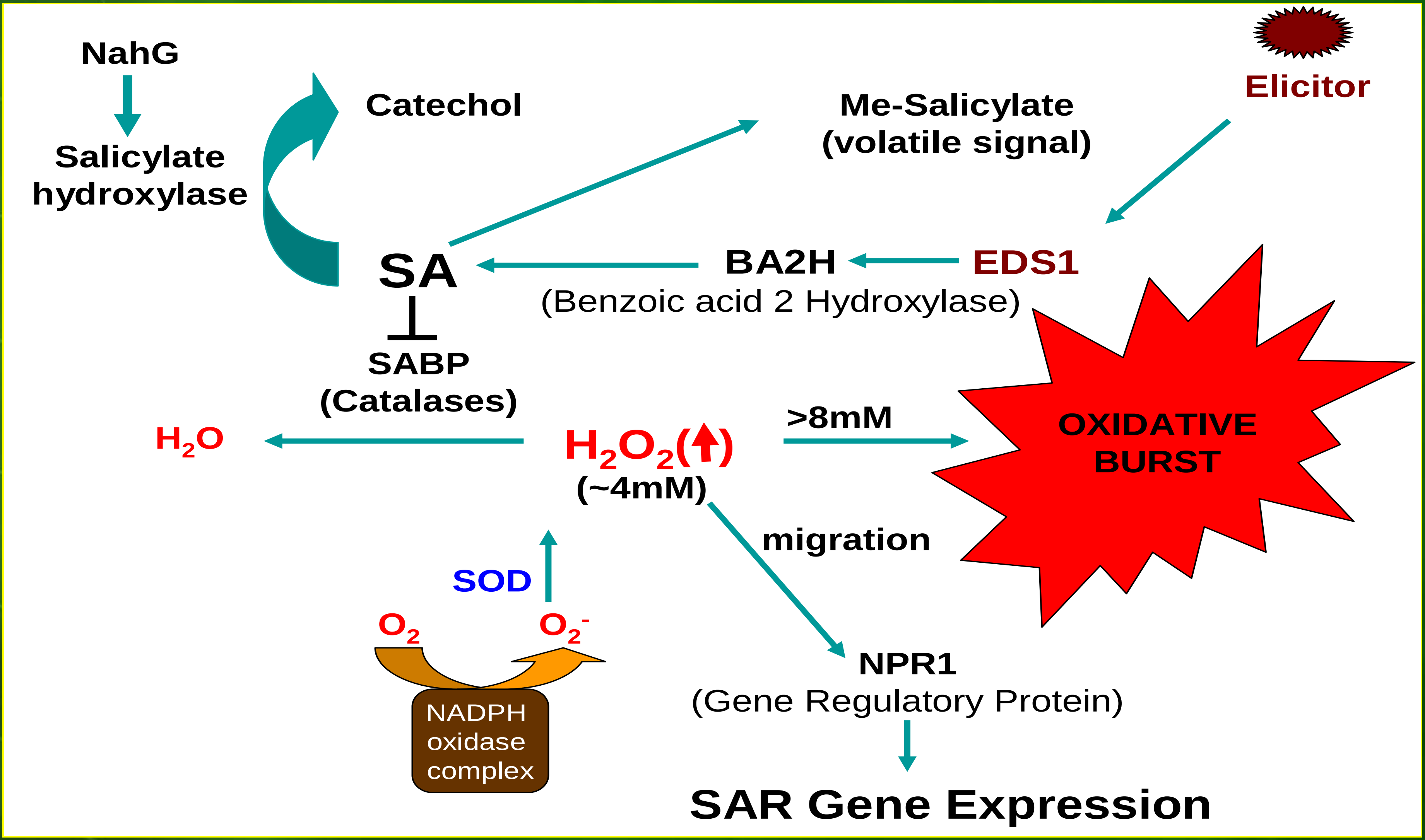
Benzo (1,2,3) thiadiazole carbothioic acid-S-methyl ester (BTH)



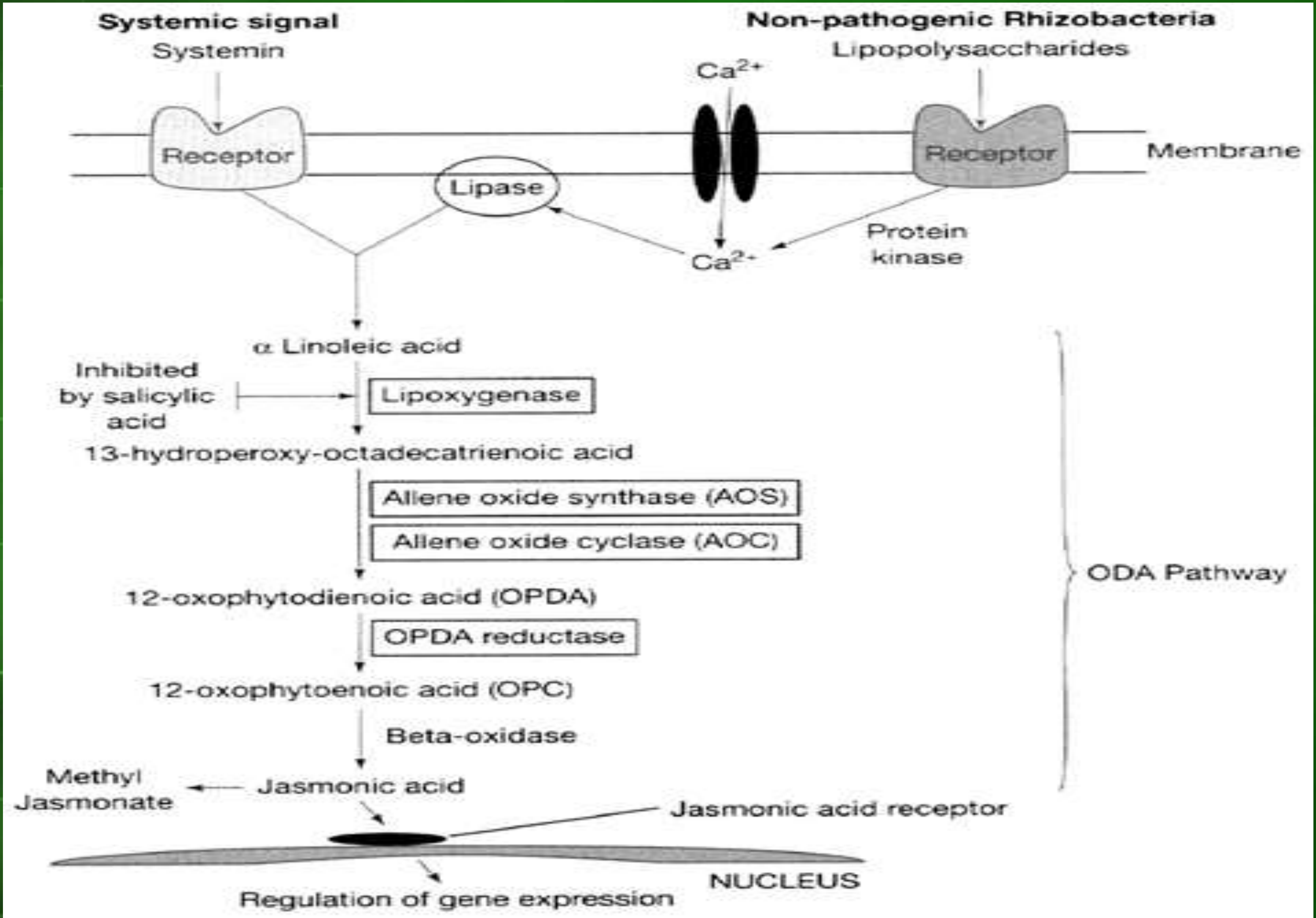
Biosynthesis of SA in plants

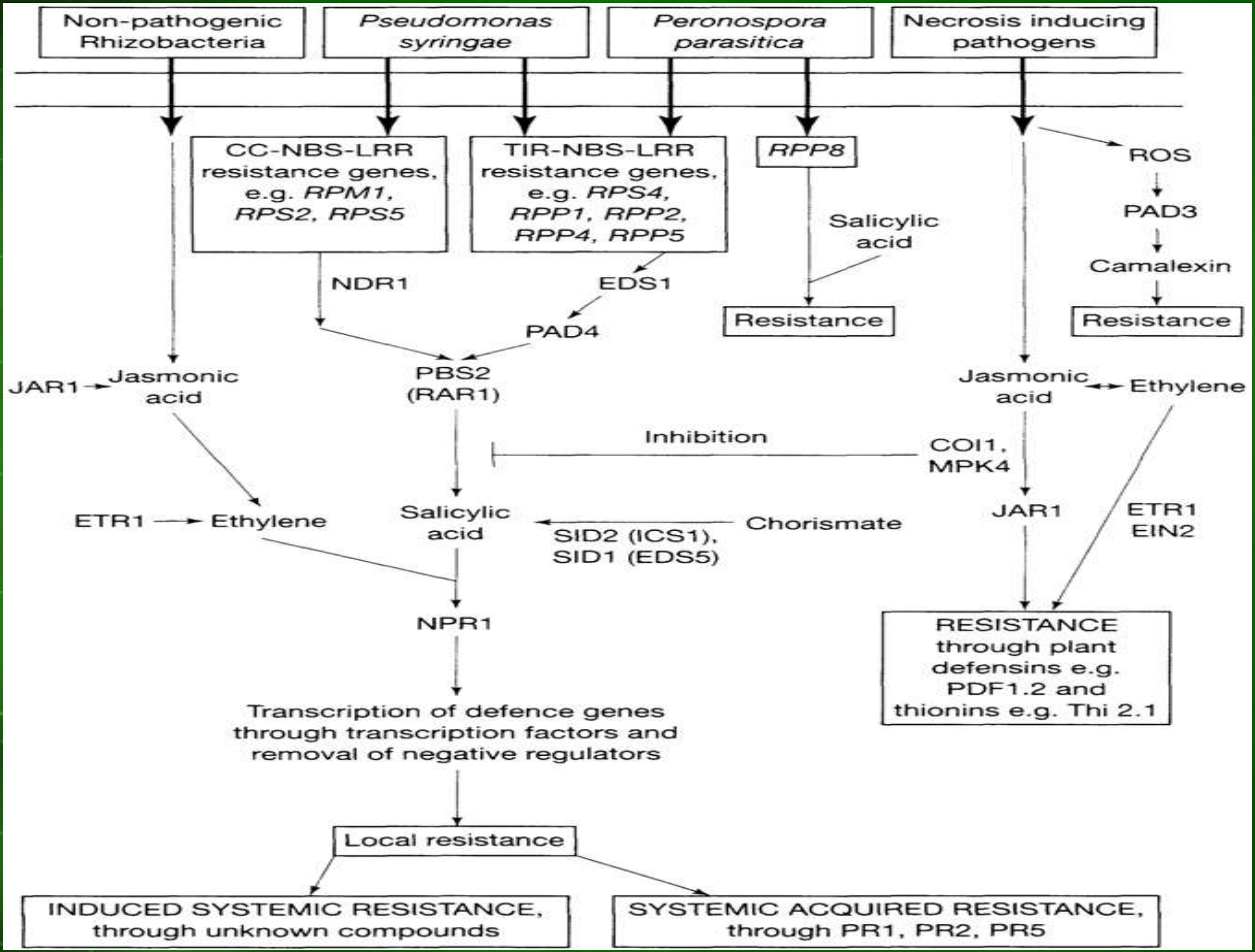


Signaling Network Of SA



The biosynthesis of jasmonic acid




Non-pathogenic Rhizobacteria
Pseudomonas syringae
Peronospora parasitica
Necrosis inducing pathogens
CC-NBS-LRR resistance genes, e.g. RPM1, RPS2, RPS5
TIR-NBS-LRR resistance genes, e.g. RPS4, RPP1, RPP2, RPP4, RPP5
RPP8
ROS
PAD3
Camalexin
Resistance
Salicylic acid
Resistance
NDR1
EDS1
PAD4
PBS2 (RAR1)
JAR1
Jasmonic acid
Jasmonic acid
Ethylene
Inhibition
COI1, MPK4
ETR1
Ethylene
Salicylic acid
Chorismate
SID2 (ICS1), SID1 (EDS5)
JAR1
ETR1 EIN2
NPR1
RESISTANCE through plant defensins e.g. PDF1.2 and thionins e.g. Thi 2.1
Transcription of defence genes through transcription factors and removal of negative regulators
Local resistance
INDUCED SYSTEMIC RESISTANCE, through unknown compounds
SYSTEMIC ACQUIRED RESISTANCE, through PR1, PR2, PR5

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