PL PATH 604

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Plant continuously defend themselves against attack from fungi, bacteria, viruses, invertebrates, etc.

Do not have immune system.

Rather possess preformed and inducible defense resistance.

Plant disease resistance often depends on ability of recognition signals activation to initiate response.
For successful establishment of pathogenicity steps are:

- Attachment to the plant surface
- Germination on the plant surface and formation of infection structures
- Penetration of the host
- Infection of the host
- Colonization of the host

Plant pathogen interaction is generally susceptible if the fungus produces characteristics symptoms or resistance if plant resist symptoms development and pathogen reproduction.
Compatible interaction

(Agrios, 2007)
Incompatible interaction

(Agrios, 2007)
Recognition - the first step in interaction.

If the initial recognition signal received by pathogen favors its growth and development – outcome-Disease.

If signal suppresses pathogen growth and activity, disease will aborted.

Recognition depends on generation of elicitors by the pathogen.
Recognition of pathogen triggers a large range of inducible defense mechanisms. Leads to resistance in plants. Various mechanism induced at site of infection like synthesis of antimicrobial compounds called phytoalexins, alteration in synthesis of cell wall structural proteins and response is generated which is involved in defense signal transduction.

(Agrios, 2007)
Biologically, signal transduction refers to any process by which a cell converts one kind of signal or stimulus into another.

Signal transduction at the cellular level refers to the movement of signals.
Steps of Signal Transduction:

- Signal perception
- Signal transduction
- Signal response
- Termination of signaling events
Generalized view of signal transduction

Plant pathogen-interactions

Pathogen

elicitor

Pathogenicity
factors

Cell wall

Plasmalemma

Defence response

Product cell wall modifications
phytoalexins
PR-proteins
HR & systemic signals

Host
genomics
cDNA

Signal transduction

transcription

mRNA

translation

Antisera - peptide seq

(Martin, 1999)
Signal Perception

- It is a surface level phenomena in which elicitor from the pathogen are recognized by host receptor.

- Explained by Flor gene-for-gene hypothesis and consist of a receptor-ligand interaction.
**Gene-for-gene hypothesis**

For each gene that determines resistance in the host, there is a corresponding gene in the pathogen that determines (a)virulence.

- Harold Flor (1950s)

(Flor, 1956)
The perception of signals from plant surfaces by pathogenic fungi seems to be the result of signaling pathways mediated by cyclic adenosine monophosphate (cAMP) and mitogen-activated protein kinase (MAPK), which have been implicated in regulating the development of infection-related phenomena in many different fungi.

- **Appressorium formation**

**Cyclic adenosine monophosphate (cAMP)**

- Transmission of the cAMP signal proceeds via the cAMP-dependent activity of protein kinase A (= PKA) and subsequent phosphorylation of target proteins.
- The major activity of PKA in developing germ tubes is the mobilization of carbohydrates and lipids to the appressorium site and is, therefore, pivotal to the production of functional appressoria.
- In some fungi, cAMP signaling is required for the Initiation of appressorium development, at which time intracellular cAMP concentrations rise during differentiation of conidia and emergence of the appressorium germtube. Subsequently, cAMP levels fall as the germ tube extends
- and, if more cAMP is added at this point, further development of the germ tube is inhibited.
Mitogen-activated protein kinases (MAPKs)

- Signaling pathways for infection-related development are also achieved through mitogen-activated protein kinases (MAPKs) and their upstream regulatory kinases.
- All of these together comprise a functional unit that transmits input signals from the periphery of the cell to the cell nucleus to elicit the expression of appropriate genes.
- A MAP kinase, K1 or P1, regulates appressorium formation in response to a signal from the plant surface but it is also required for invasive growth or viability in its host plant.
Elicitors released during pathogen attack recognized by receptor of the plasma membrane.

(Pollard and Earnshaw, 2002)
After perception next step is signal transduction. Perception of environmental signals, mediated by specific receptors, likely initiates internal signal pathways.

There are two major pathways by which signal can be transduced i.e. Via protein kinases & Via G-protein.

Detection of signal by the host receptor activates the signal transduction pathways.
Signal Transduction Pathways

Refers to a series of sequential events, such as protein phosphorylations, consequent upon binding of ligand by a transmembrane receptor, that transfer a signal through a series of intermediate molecules until final regulatory molecules, such as transcription factors, are modified in response to the signal.
G-Proteins and Cyclic Nucleotides

- Guanine nucleotide binding proteins regulate a variety of physiological processes, including sensual perception, protein synthesis, hormonal regulation, vesicular and nuclear transport, cell growth and differentiation. This superfamily includes members of small monomeric Ras-related proteins, the heterotrimeric G-proteins and the factors involved in protein synthesis. They act as molecular mediators,

- G-proteins are composed of three subunits: $\alpha$, $\beta$, $\gamma$, where specificity mainly determined by $\alpha$. The $\alpha$-subunit consists of two domains: GTPase domain and $\alpha$-helical domain. Activation results in conformational changes around so called switch I, II and III regions in GTPase-domain.
G Proteins and cyclic nucleotides
cAMP- second messengers of intracellular events
- receptors- stimulates conversion of ATP to cAMP associated with G proteins
- binding with membrane bound receptor leads to activation of α-subunit of G protein
- Gs-stimulates, Gi-inhibits–Adenylnyl cyclase (AC)
- AC stimulation- catalyze ATP to cAMP conversion
- activation cAMP- dependent protein kinase A –PKA
- PKA phosphorylates protein- intracellular enzyme
- either increase or decrease the activity

(Francis and Corbin, 1999)
Cyclic Nucleotide Metabolism - cGMP

- cGMP - second messenger of intracellular events
- Peptide hormones activate receptors associated with membrane-bound guanylate cyclase (GC)
- Leads to conversion - GTP to cGMP
- NO- stimulates cGMP production by activating soluble GC
- cGMP- mediates intracellular effects by activation of specific cGMP dependent protein kinases (PKG)
- PDE catalyzes the degradation of cGMP to guanosine-5’-monophosphate (5’-GMP)
-PKG phosphorylate protein-intracellular enzyme

(Receptor)
Various second messenger system regulated by g-protein activation:

- Adenylyl cyclase
- Phospholipase C
  - Inositol Triphosphate (IP3)
  - Diacylglycerol (DAG)
- Ion channel activity
- The G-protein involves in most signaling processes
- Heterotrimers: α, β and γ subunits

(Schoneberg, 1999)
Protein Phosphorylation
Mitogen-activated Protein Kinase (MAPK) Cascades

MAPK cascades fulfill essential functions in transduction of extracellular signals to cellular and nuclear responses.

MAPK is activated by dual phosphorylation of a threonine catalyzed by MAPK kinase (MAPKKK).

MAPKKs activated by serine/threonine phosphorylation by a MAPKK kinase (MAPKK).

All MAPK pathways operate through sequential phosphorylation events to phosphorylate transcription factors and regulate gene expression.
Ca$^{2+}$ binding and ca$^{2+}$ dependent pathways

- Ca$^{2+}$ -important second messenger.
- Regulatory functions of Ca$^{2+}$ ion exerted mostly by the small cytoplasmic protein calmodulin.
- On binding Ca$^{2+}$, calmodulin undergoes a major conformational change- allows it to bind to calmodulin-dependent enzymes.
- Calmodulin-activated protein phosphatase and the calmodulin-dependent protein kinase (CDPK).
- Small change in cytoplasmic Ca$^{2+}$ concentrations ‘switch on’ the active form of calmodulin.
Ion Fluxes

- Ion fluxes occurs across plasma membrane.
- Include efflux of $K^+$ ions and influx of $Ca^{2+}$. 

(Spiegel, 1996)
Secondary Messengers

- Secondary messenger system exists in plants to transmit the primary elicitation signal of pathogen and/or host.
- These are:
  - Calcium ion influx
  - Protein phosphorylation
  - cAMP
  - Active oxygen species
  - Salicylic acid
  - Methyl Jasmonic and Jasmonic Acid
  - Ethylene
  - Nitric Oxide
Active oxygen species

- Several active oxygen species (AOS) involved in signal transduction like:
  - $\text{H}_2\text{O}_2$
  - Superoxide anion ($\text{O}_2^-$)
  - Singlet oxygen ($^{1}\text{O}_2$) and
  - Hydroxyl radical (OH) (Du and Klessig, 1997)
- $\text{H}_2\text{O}_2$ is the most important second messenger
Salicylic Acid

Salicylic acid (SA) is a phenolic compound commonly present in plant kingdom.

One of the most important signal molecules, acts locally in intracellular signal transduction.

SA suppress the $\text{H}_2\text{O}_2$ degrading activity of catalase (Conrath et al., 1995)

Salicylic acid (SA) is an essential signal molecule for the onset of SAR.
Methyl Jasmonic and Jasmonic Acid

- Naturally occurring compounds in plants
- JAs affect a variety of physiological processes and mediate plant responses to stresses by pathogen (Farmer and Ryan, 1992).
- Low concentration induce different enzymes involved in plant defense
- Phenylalanine ammonia-lyase (PAL) and lipoxygenase.

(Okada, 2009)
Ethylene

- Ethylene is a volatile plant hormone
- Synthesized from amino acid methionine.
- Ethylene is produced upon wounding or infection by pathogen as well as by treatment by elicitors of defense responses (Boller, 1990).
• The increased production of ethylene is one of the earliest chemically detectable events in pathogen-infected plants or treated plants with elicitors (Toppan and Tugaye, 1984).

• **ISR is commonly regulated by** jasmonic acid (JA)- and ethylene (ET)-dependent signaling pathways.
Nitric Oxide

- NO signaling involves cyclic GMP-dependent pathways.
- NO signaling in tobacco requires cGMP synthesis (Durner et al., 1998).
- NO activates MAP kinases in tobacco (Kumar and Klessig, 2000) and Arabidopsis.
- Biosynthesis of NO is catalyzed by nitric oxide synthase (NOS) enzyme.
- ROS known to work with nitric oxide (NO) in defense responses.
Massive changes in gene expression

Plants response to pathogen infection is associated with massive changes in gene expression

In *Arabidopsis* more than 2000 genes changed expression levels within 9 h of inoculation with the pathogen *Pseudomonas syringae* (Glazebrook *et al.*, 2003).
The signal should terminate when it is induced and responded to.
The interaction between plant and pathogen are specific, complex and dynamic.

Signals for activation of various defenses initiate in response to recognition.

The outcome of interaction dependent on initial sensing of the other organism via exchange of molecular signal through signaling cascade and modified gene expression.

Recognition is the first step by which response is generated which is involved in defense signal transduction.
Secondary messenger system required in plants to transmit the primary elicitation signals.

Many secondary molecules are involved in signal transduction process.

Signaling outcome leads to massive changes in gene expression.