



Hrp-Avr Genes in Plant Bacterial Interactions

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Abstract

Hrp genes control the ability of phyto-pathogenic bacteria to cause disease and to elicit hypersensitive reactions on resistant plants. Genetic and biochemical studies have demonstrated that Hrp proteins are components of Type III secretion systems, regulatory proteins, proteinaceous elicitors of the hypersensitive reaction, and enzymes needed for synthesis of periplasmic glucans. Significantly, Type III secretion systems are involved with the secretion of pathogenicity proteins in bacterial pathogens of animals. The transcriptional activation of a number of bacterial avirulence (avr) genes is controlled by Hrp regulatory proteins, and recent experimental evidence suggests that Avr proteins may be transported by Hrp secretion systems. It has also been hypothesized that pathogenicity and/or virulence gene products exit bacterial phytopathogens via Hrp pathways. Thus, hrp genes may be one of the most important groups of genes found in phytopathogenic bacteria in relationship to pathogenicity and host range.

Keywords- hrp avr genes, type III secretion system, avirulence

Introduction

Gene for gene hypothesis

For each gene of resistance in host there must be a gene for avirulence in pathogen and vice-versa

- In the 1940s, Flor developed the gene-for-gene concept to explain the genetic interactions between *Melampsora lini* and *flax*. His theories were put to use in his own flax breeding program to successfully develop rust-resistant flax.
- This concept provided the underpinnings for research on the genetics of host-pathogen

interactions.

Avirulence (avr) genes

- First identified by H. H. Flor in late 1940s.
- One of the elicitors of plant defense response.
- Involved in determining compatibility b/w host and pathogen.
- The *avr* genes make a pathogen avirulent i.e., warn the host and inhibits the pathogen.
- They have also been found essential for virulence in some pathogens like *pthA* gene of *X. citri* • (Alfano, et al., 2016).
- **Race specificity of *avr* genes**
- *avr* genes impose race – specificity on the pathogen i.e., compatible association with a plant species/cultivar.
- Eg; *avrA* gene of race 6 strain of soyabean pathogen (*P. s. pv. glycinea*) is able to elicit defense responses only on cultivars with the *Rpg2* gene for resistance

Organisation of *avr* genes

- *avr* genes are located on plasmids of bacteria.
- Monocistronic with single open reading frame (ORF).
- *avr* genes products are hydrophilic and contain multiple copies of signal peptide sequence.
- E.g; *pth* gene of *Xanthomonas* codes a protien that has 13-20 copies of naerly identical 34 amino acid repaet unit.

***hrp* genes**

- Hypersensitive reaction and pathogenicity genes
- First identified in *P. syringae pv. Phaseolicola*, a pathogen of bean.
- Identified in all gram negative bacteria (except *Agrobacterium*). E.g.
Pesudomonas syringae, *Xanthomonas campestris*,
Ralstonia solanacearum, *Erwinia amylovora*.

Location of *hrp* genes

- *hrp* gene clusters are presumably located on the chromosome of the bacteria.
- However, in *R. solanacearum* it is found on 200- Kb megaplasmid.

Genetic Organisation of *hrp* Gene Clusters

- *hrp* gene clusters are physically and functionally conserved among strains of phytopathogenic bacteria.
- *hrp* genes are organised in large clusters consisting of multiple transcriptional units.
- Some genes are found in genomic regions that are distinctly far from *hrp* clusters. (*hrpM*, in *P.s.pv.phaseolicola*)

Regulation of *hrp* genes

- The *hrp* genes are not expressed in conditions which favour bacterial growth.
- They are expressed only under stress conditions (Huang *et al.*, 2022).
- Affected by various carbon and nitrogen sources, pH, osmolarity, temperature and plant signals.
- Environmental signals that induce *hrp* gene expression are specific for each bacterium.

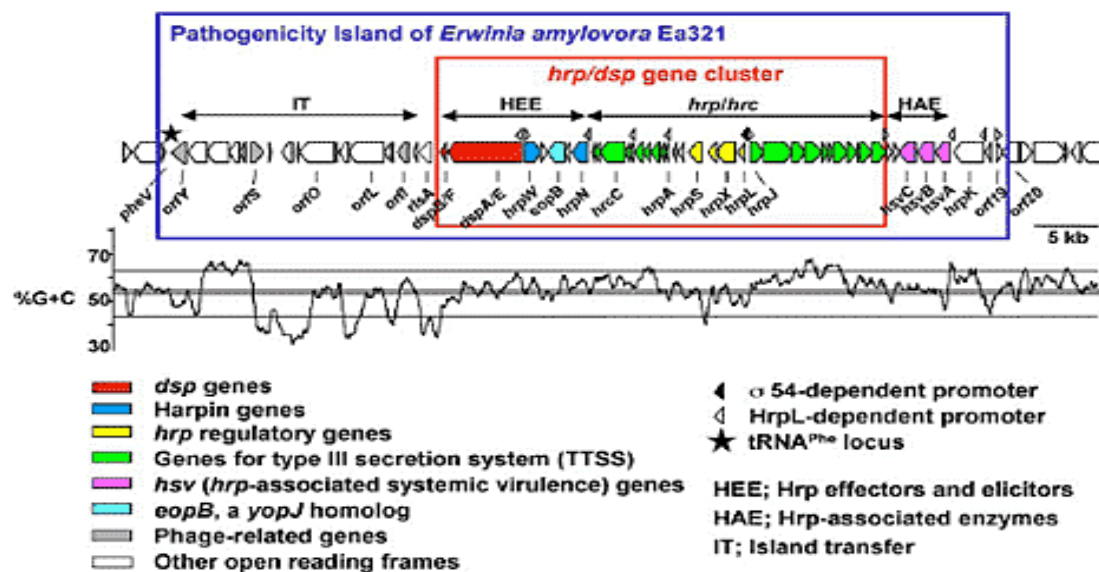


Fig 1. Genetic Organisation of *hrp* Gene Clusters

Functions of *hrp* genes and its products

- *hrp* gene clusters encode 19-26 unique protein products.
- *hrp* genes are involved in early stages of establishment of the parasitic relationship.
- These products are involved in specific biochemical functions such as:
 1. *Hrp* regulatory function
 2. Major components of Type III Secretion System
 3. β -(1,2)-Glucan Synthesis.
 4. Increasing population
 5. Elicitors of HR (*Harpins*).

hrp Regulatory systems

- *hrp* clusters contain genes involved in transcriptional regulation of *hrp* and other genes.
- In *R. solanacearum*, *hrpB* encodes one of the key regulatory genes controlling pathogenicity functions: activated by environmental stimuli.
- HrpB in turn induces the expression of at least five operons, including the TTSS genes and some of the Hrp-dependent substrates.

Model for *hrp* gene regulation and type III secretion in *X.campestris* pv. *vesicatoria*

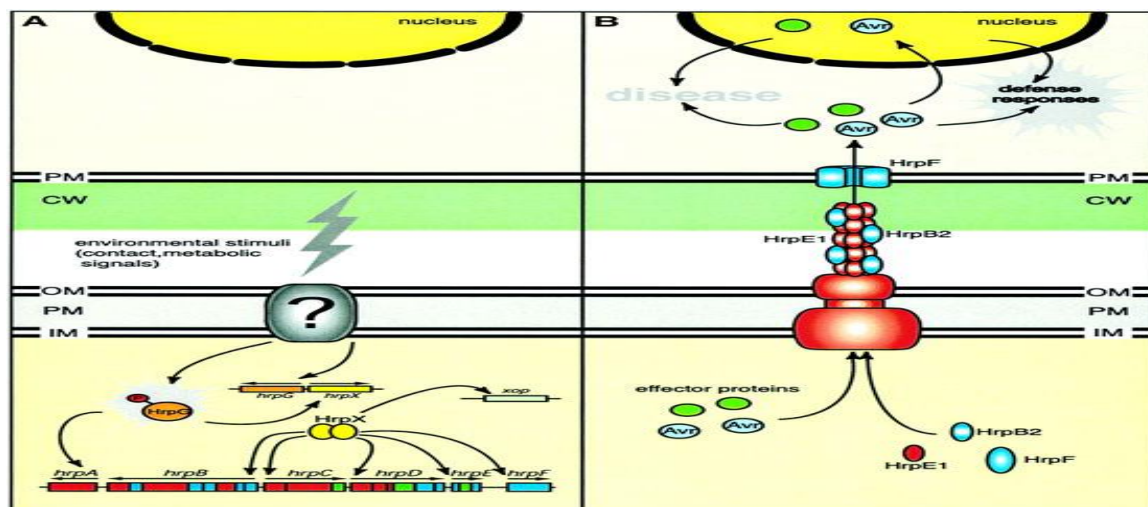


Fig 2. Model for *hrp* gene regulation and type III secretion in *X.campestris* pv. *Vesicatoria*
(Vanneste *et al.*, 2019).

- TTSS is a set of 15 – 20 proteins..
- In association with bacterial membrane, makes up secretion apparatus.
- These translocate host-specific ‘effector’ proteins from bacteria into the host plant cells.
- Chaperons of TTSS bound effectors and prevent premature interactions with other proteins.

Elicitors of HR:

- *Harpins* – *hrp* Dependent elicitor proteins. (HrpN = harpin) (Wei *et al.*, 1992)
- Produced by *hrp* gene clusters.
- Differ from *Avr* genes in that they do not require R genes for determining resistance.
- They associate with liposomes and with bilayer membrane, form pores; water and nutrients move out of the cell to the apoplast.
- These pores also serve as openings for other effector proteins.
- PoPA1 is a glucan rich molecule regulated by *hrpB*, elicits HR in tobacco and petunia plants.
- PoPA1 is similar to *Harpins*.

These play no role in pathogenicity.

Hrp–avr Interaction

- *avr* genes are dependent on functional *hrp* genes.
- *avr* genes show co-ordinate expression with *hrp* genes i.e., expressed in response to *hrp* regulatory factors.
- A number of genes, including *avrD*, *avrB*, *avrPto*, *avrRpt2*, are induced upon expression of the transcriptional regulator loci *hrpS* and *hrpL*.
- In the absence of the regulatory genes, most *avr* genes would not be induced.

Harpins

- Harpin acts by eliciting a complex natural defense mechanism in plants, analogous to a broad spectrum immune response in animals.

- Harpin elicits a protective response in the plant that makes it resistant to a wide range of fungal, bacterial, and viral diseases.
- Harpin protein has the potential to substantially reduce use of toxic pesticides, especially fungicides and certain soil fumigants, such as methyl bromide.
- Application of Harpin in small quantities effectively activates plant defense response without eliciting any visible HR (Wei *et al.*, 2012).
- Harpins are glycine-rich and heat-stable proteins that are secreted through type III secretion system in gram-negative plant-pathogenic bacteria.
- Wei *et al.* 1992 characterized HrpN gene product of *Erwinia amylovora* as the first pathogen independent HR elicitor in plants and protein was given the name Harpin (encoded by hrpN).

Conclusion

Cloning the first avirulence (avr) gene has led not only to a deeper understanding of gene-for-gene interactions in plant disease, but also to fundamental insights into the suppression of basal defences against microbial attack. Bioinformatics-led analysis of effector gene distribution in genomes has provided a remarkable view of the interchange of effectors and also their functional domains, as the arms race of attack and defence drives the evolution of microbial pathogenicity.

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