Genome Editing and Engineering

Course No: BT-637

LECTURE-13

Dr. Kusum K. Singh
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati

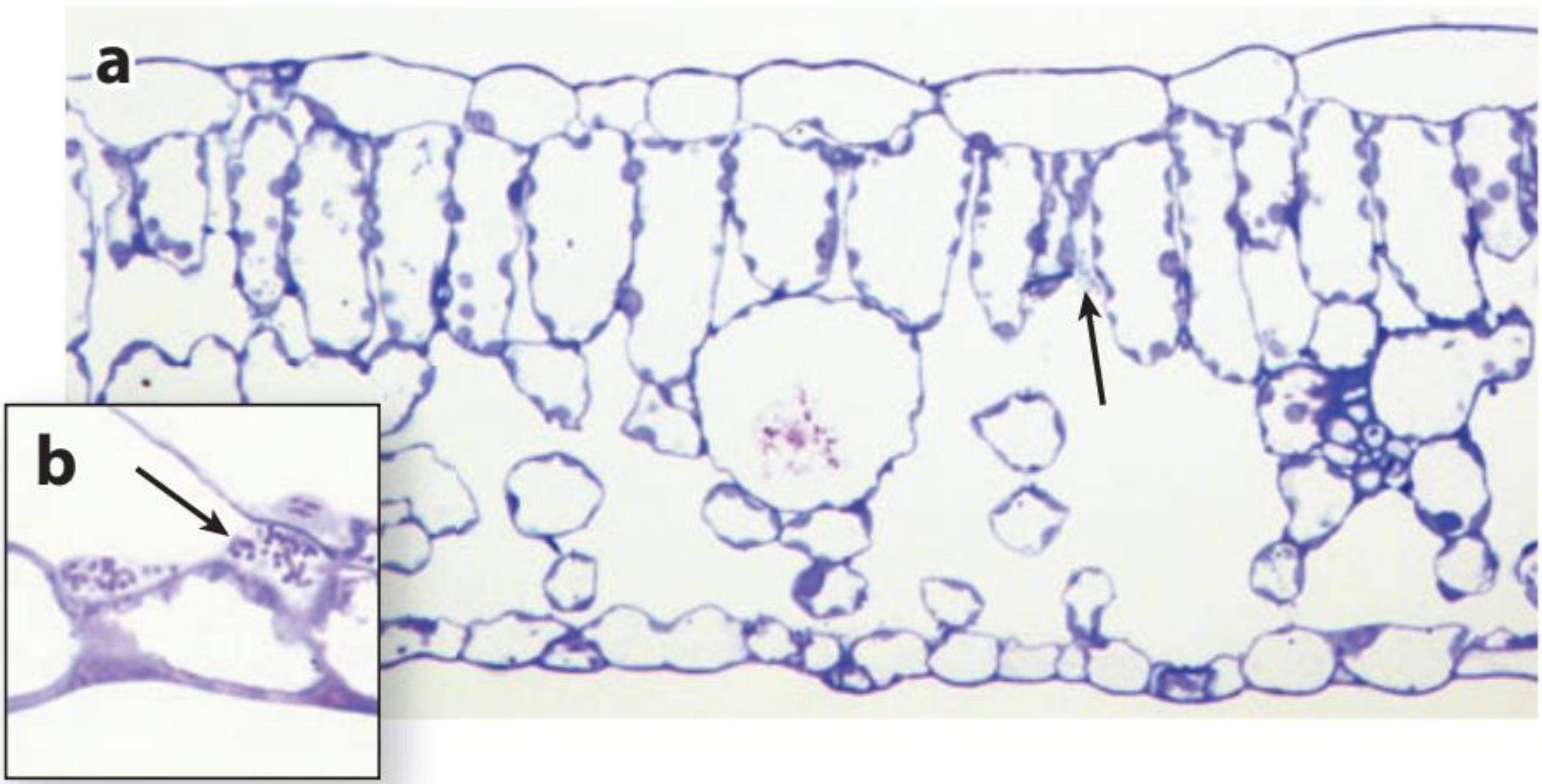
Gram negative “Xanthomonas”

Xanthomonas sp.
Gram negative

Species or pathovar	Strain[a]	Host	Location	Serovar	Pattern
Pseudomonas solanacearum	ORST 1153 b	*Solanum melongena*	Congo		1
	ORST 1153 2c	*Solanum melongena*	Congo		1
	ORST 1155 2a	*Solanum melongena*	Congo		1
	1000	*Lycopersicon esculentum*	French Guiana		1
Xylophilus ampelinus	CFBP 2098	*Vitis vinifera*	France		2
	NCPPB 2220	*Vitis vinifera*	Greece		2
	NCPPB 3026	*Vitis vinifera*	Italy		2
Xanthomonas fragariae	CFBP 2157	*Fragaria* sp.	United States		3
Xanthomonas axonopodis	NCPPB 2375	*Axonopus scoparius*	Colombia		4
	NCPPB 457	*Axonopus scoparius*	Colombia		4
Xanthomonas albilineans	G 7	*Saccharum* sp.	Guadeloupe	3	5
	GP 5	*Saccharum* sp.	Guadeloupe	1	5
	HV 5	*Saccharum* sp.	Burkina Faso	2	5
	R 8	*Saccharum* sp.	Réunion	1	5
	USA 083 A	*Saccharum* sp.	United States		5
	KNA 003 a	*Saccharum* sp.	St. Kitts		5
	LKA 070 A	*Saccharum* sp.	Sri Lanka		5
	G 55	*Saccharum* sp.	Guadeloupe		5
	MDG 065 A	*Saccharum* sp.	Madagascar		5
	MQE 58	*Saccharum* sp.	Martinique		5
	BF 60	*Saccharum* sp.	Burkina Faso		5
	CIV 035 A	*Saccharum* sp.	Ivory Coast		5
	2375 84	*Saccharum* sp.	Cameroon		5
Xanthomonas campestris pv.					
campestris	NCPPB 528	*Brassica oleracea*	United Kingdom		6
vesicatoria	10601	*Lycopersicum esculentum*	Undetermined		7
cassavae	HMB 29	*Manihot esculenta*	Zaire		8
vasculorum	CFBP 1289	*Saccharum* sp.	Réunion		9
juglandis	CFBP 1023	*Juglans regia*	France		10
juglandis	CFBP 1024	*Juglans regia*	France		10
phaseoli	ORST 1159	*Phaseolus vulgaris*	Congo		10
phaseoli	CFBP 1816	*Phaseolus vulgaris*	Greece		10
citri	CFBP 1814	*Citrus* sp.	Réunion		10
glycines	ORST 1144 E5	*Glycine max*	Congo		10
mangiferae indicae	CFBP 1716	*Mangifera indica*	India		11
incanae	CFBP 1438	*Matthiola incana*	United States		12
pelargonii	10342	*Pelargonium zonale*	France		15
oryzicola	CFBP 2286	*Oryza sativa*	Malaysia		13
oryzae	CFBP 1948	*Oryza sativa*	Cameroon		14

[a] Abbreviations for sources of strains: CFBP, Collection Française des Bactéries Phytopathogènes, INRA, Angers, France; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom; ORST, ORSTOM, Brazzaville, Congo. Strains of *X. albilineans* were received from P. Rott, IRAT-CIRAD, Guadeloupe, and P. Baudin, CIRAD, Montpellier, France.

Plant Pathogen



Plant Pathogen (Xanthomonas)

Plant Immunity

- "Effector-triggered" immunity
- "Pattern-triggered" immunity
- Pathogen virulence factors ("effectors")

Effector-triggered immunity in plants "gene-for gene" hypothesis

➢ **Why are certain plant varieties resistant to diseases, whereas others are susceptible?**

Harold Henry Flor

- A plant pathologist famous for proposing the **gene for gene hypothesis** of plant-pathogen genetic interaction.
- Worked on rust (Melampsora lini) of flax (Linum usitatissimum).
- He proposed term "Avirulence gene".

Plant
R gene

Pathogen
Avr gene

	+	-
+	**R: resistance**	**S: susceptible**
-	**S**	**S**

Molecular proof for the "gene-for gene" hypothesis

Plant
R gene

Pathogen
Avr gene

	+	-
+	R: resistance	S: susceptible
-	S	S

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6024–6028, October 1984
Botany

Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr.

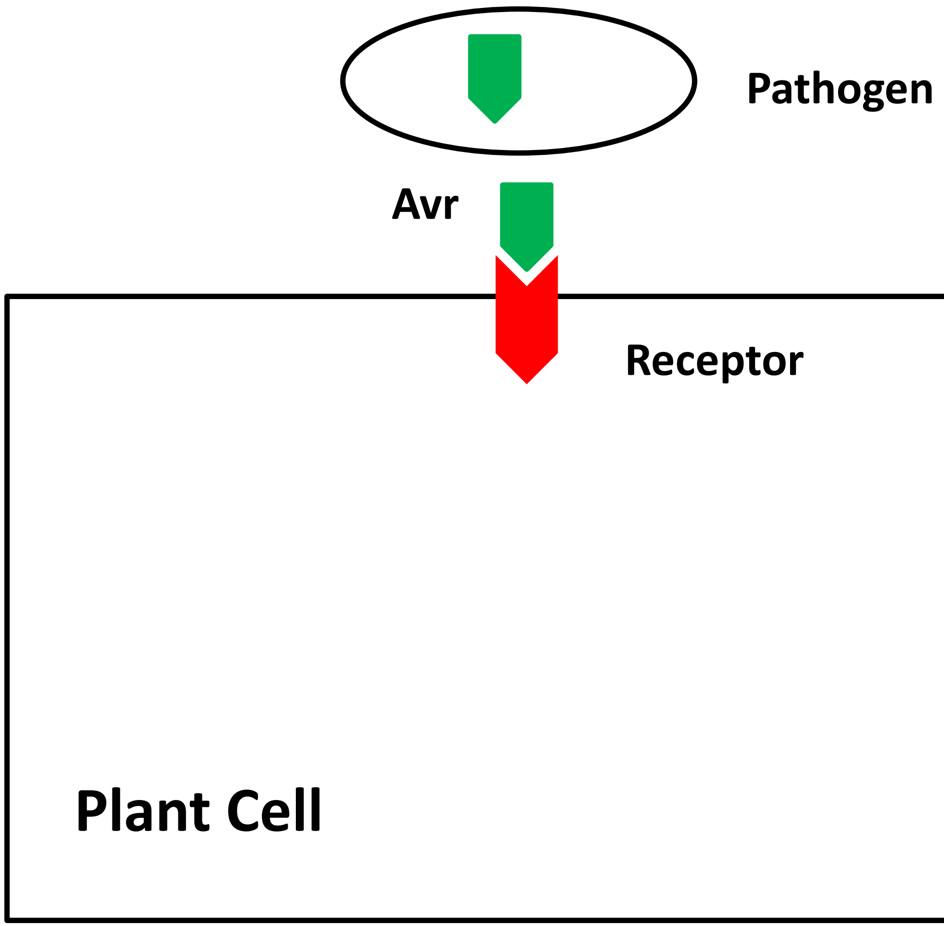
(plant-parasite interaction/disease resistance/cosmid cloning/ice nucleation)

Brian J. Staskawicz*†, Douglas Dahlbeck*†, and Noel T. Keen‡

- **Cloning of plant disease resistance genes in 1992-1994**

Many groups (S briggs, G. Martin, Tanksley B, Staskawicz, F Ausubel

Some original predictions about R and Avr proteins



Most R genes encode LRR proteins

N gene (tobacco mosaic virus)

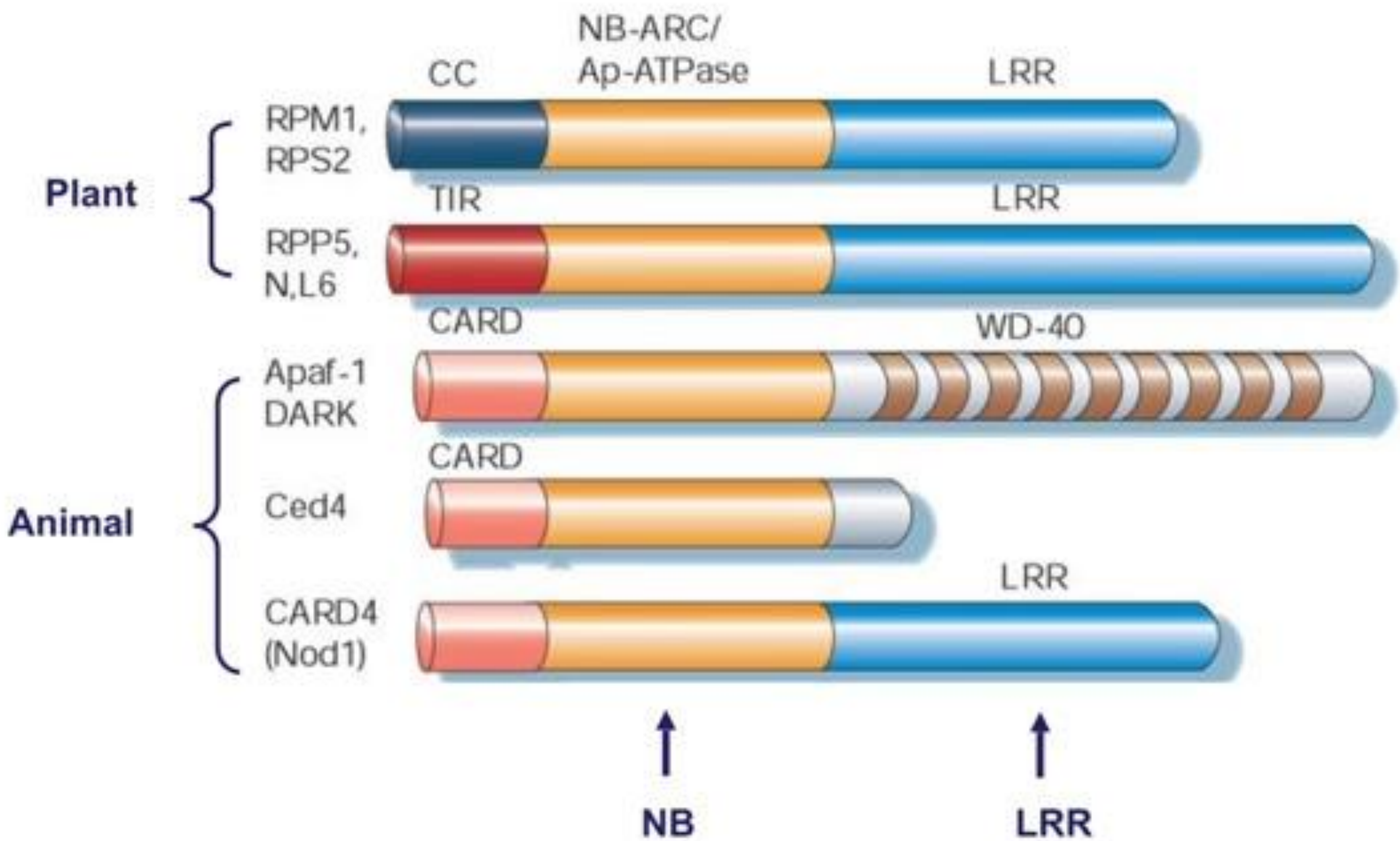
Cf9 gene (fungal leaf mold)

RPS2 gene (bacterial speck)

Mi gene (root-knot nematode)

LRR: Leucine-rich Repeats

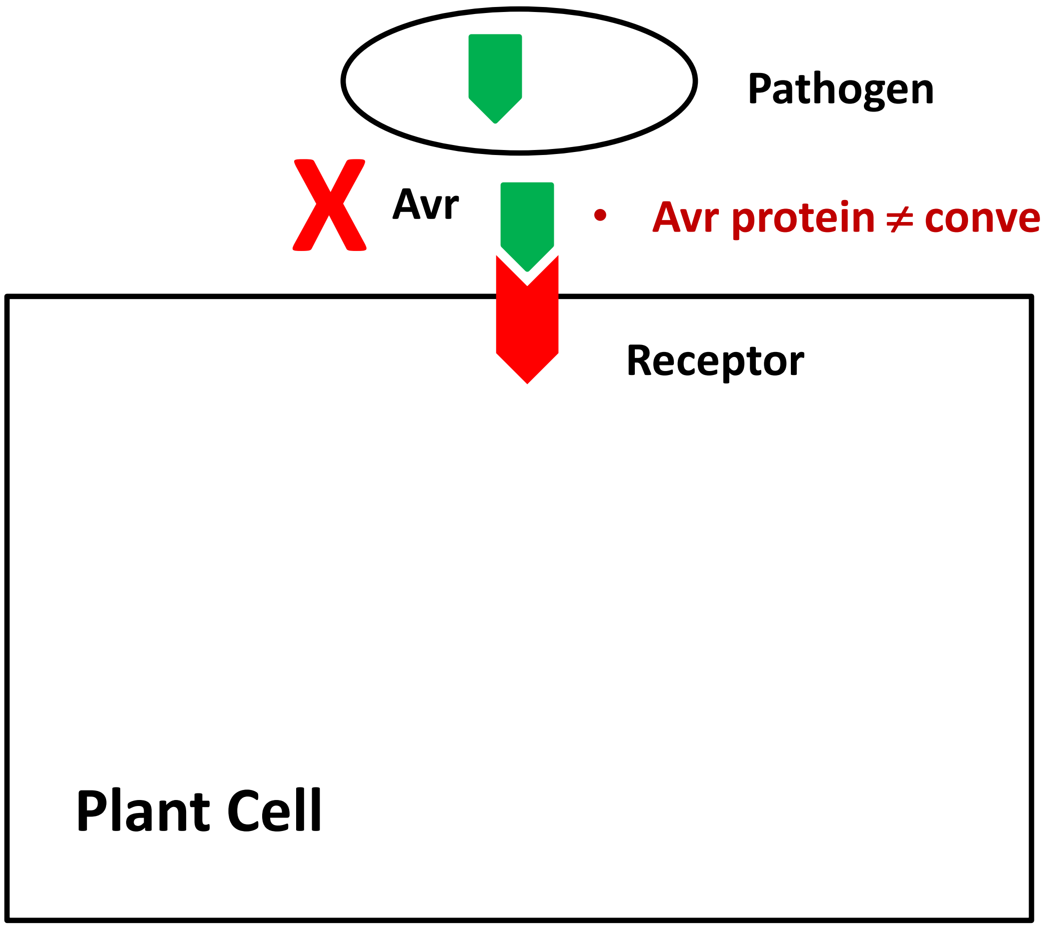
R proteins share homology with immune receptors



(NB: Nucleotide-binding)

Dangl & Jones (2001) Nature

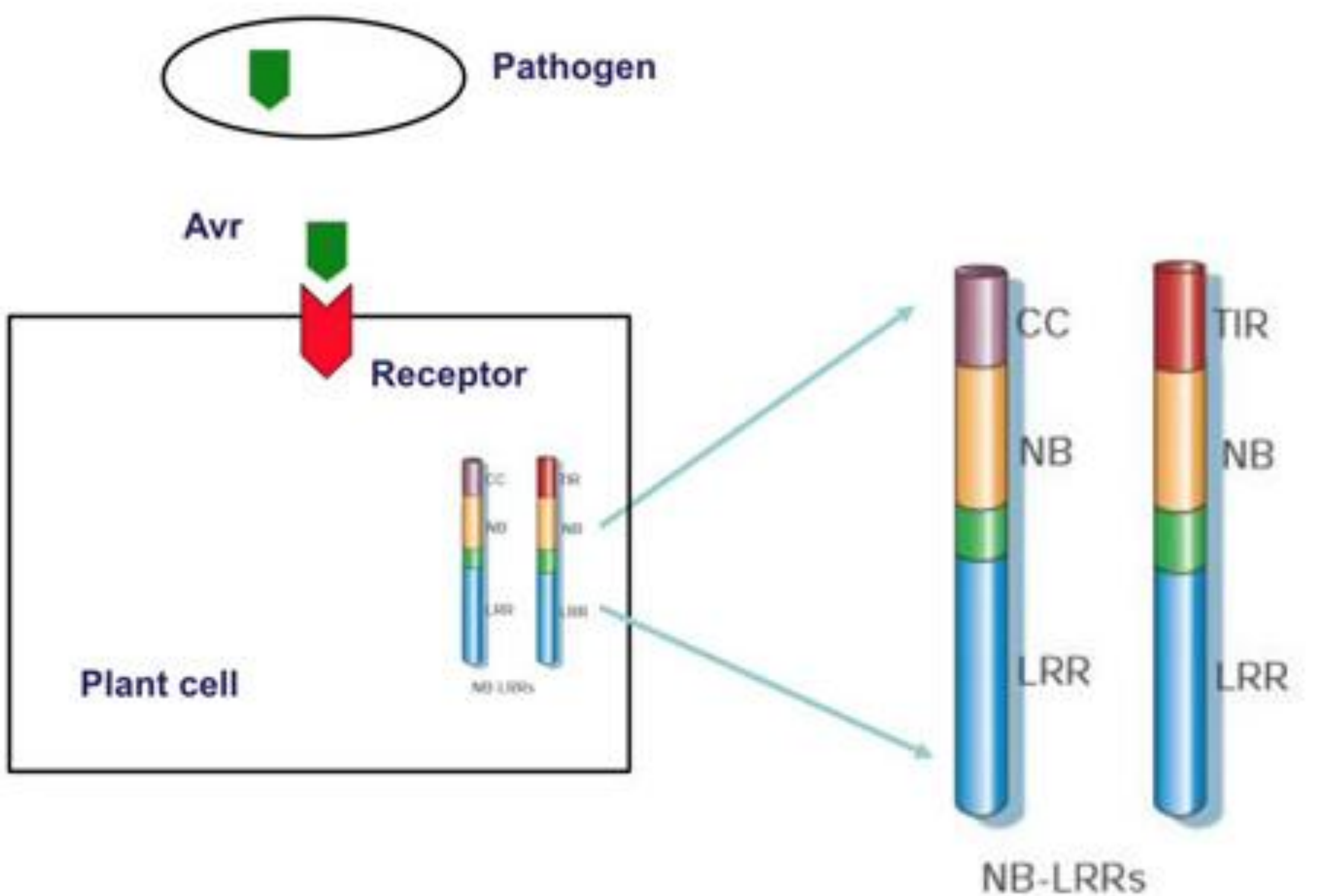
Some original predictions about R and Avr proteins



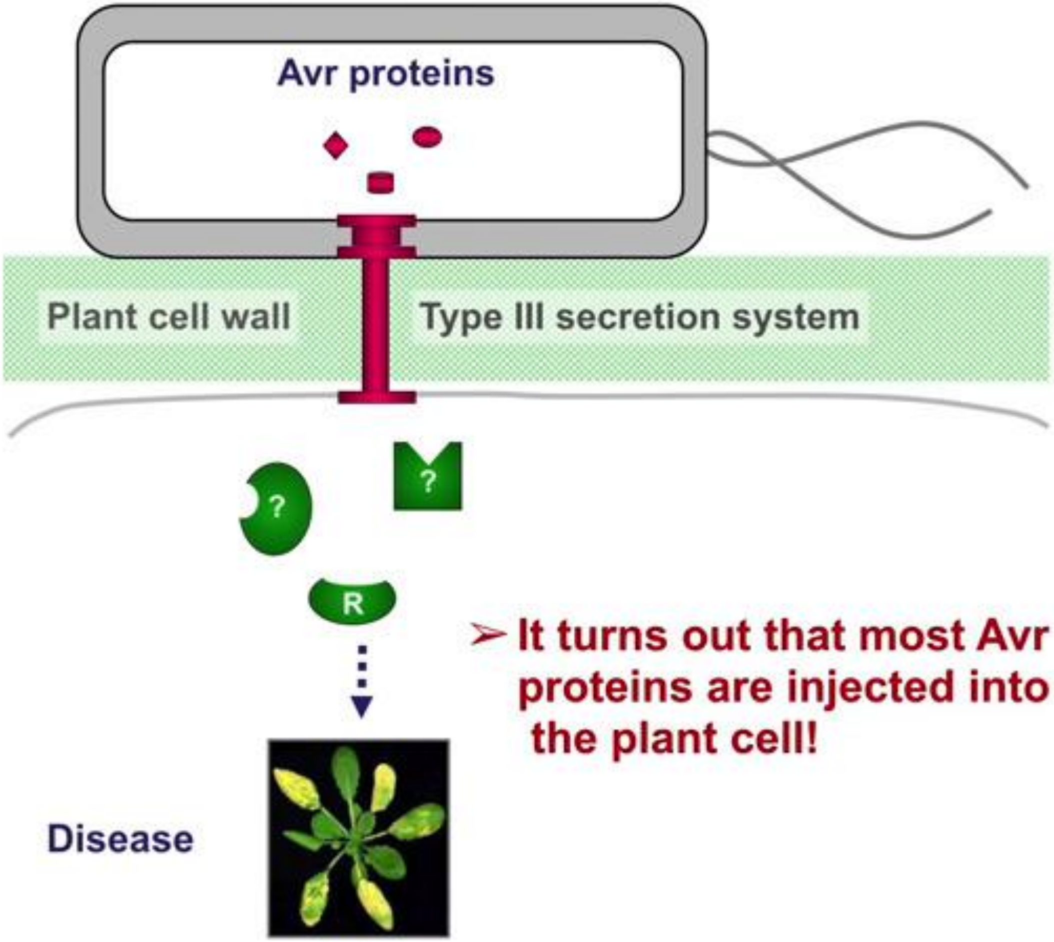
- Avr protein ≠ conventional secretion signals

Sheng-Yang He iBiology

Most R genes are localized in the cell



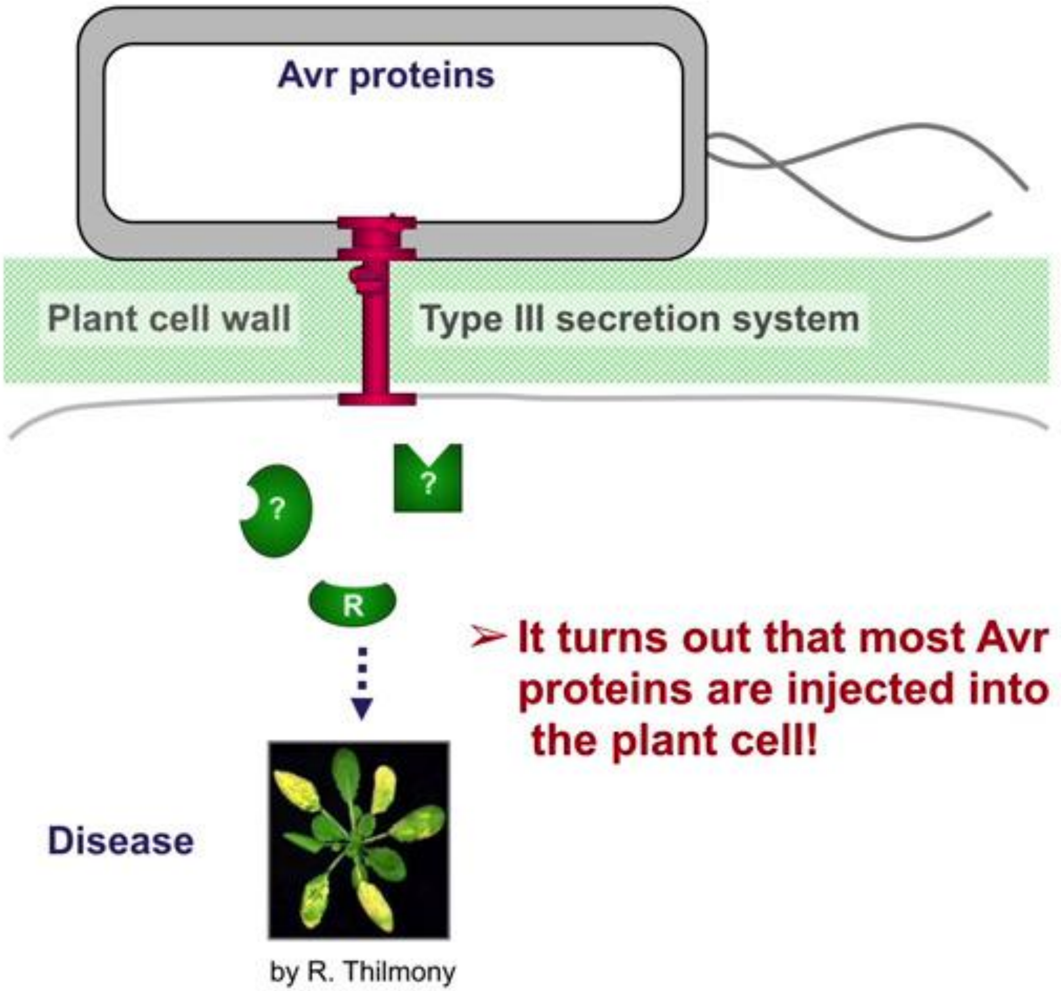
Avr is directly injected into the cells



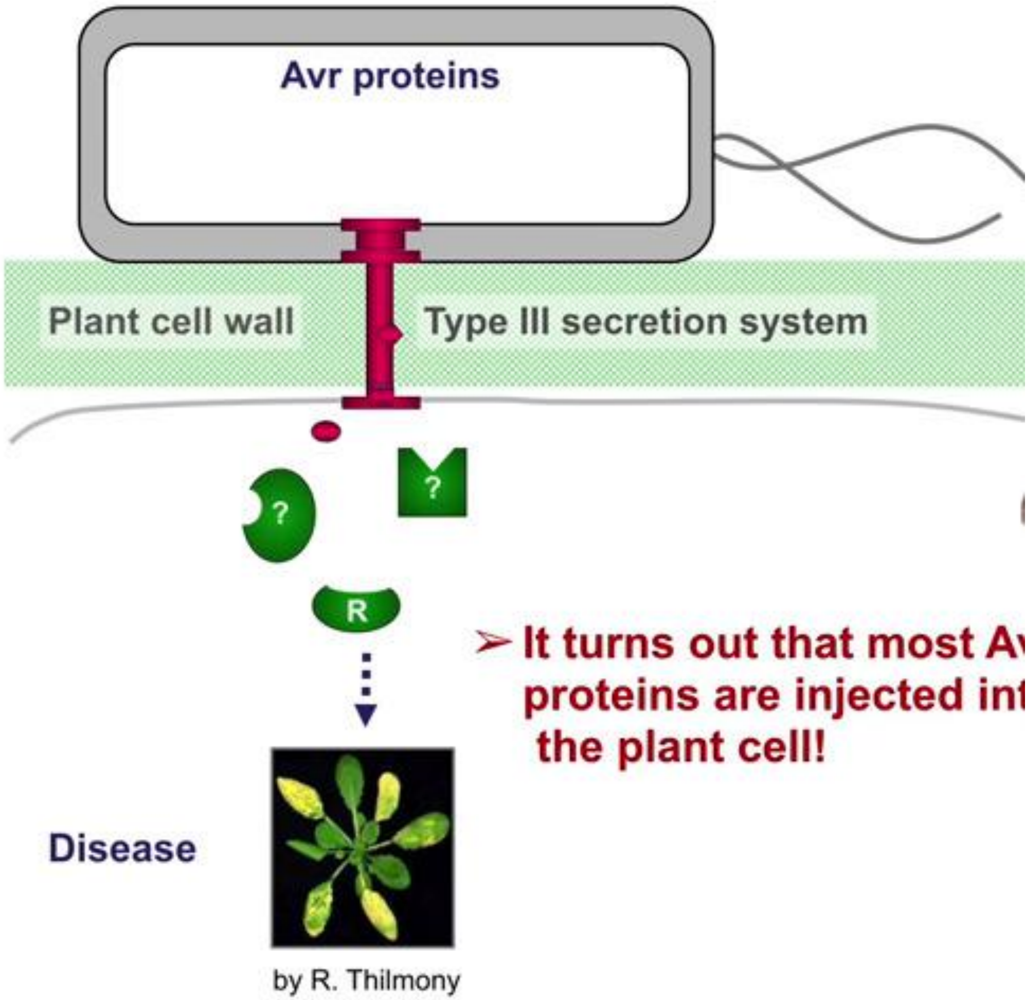
by R. Thilmony

- It turns out that most Avr proteins are injected into the plant cell!

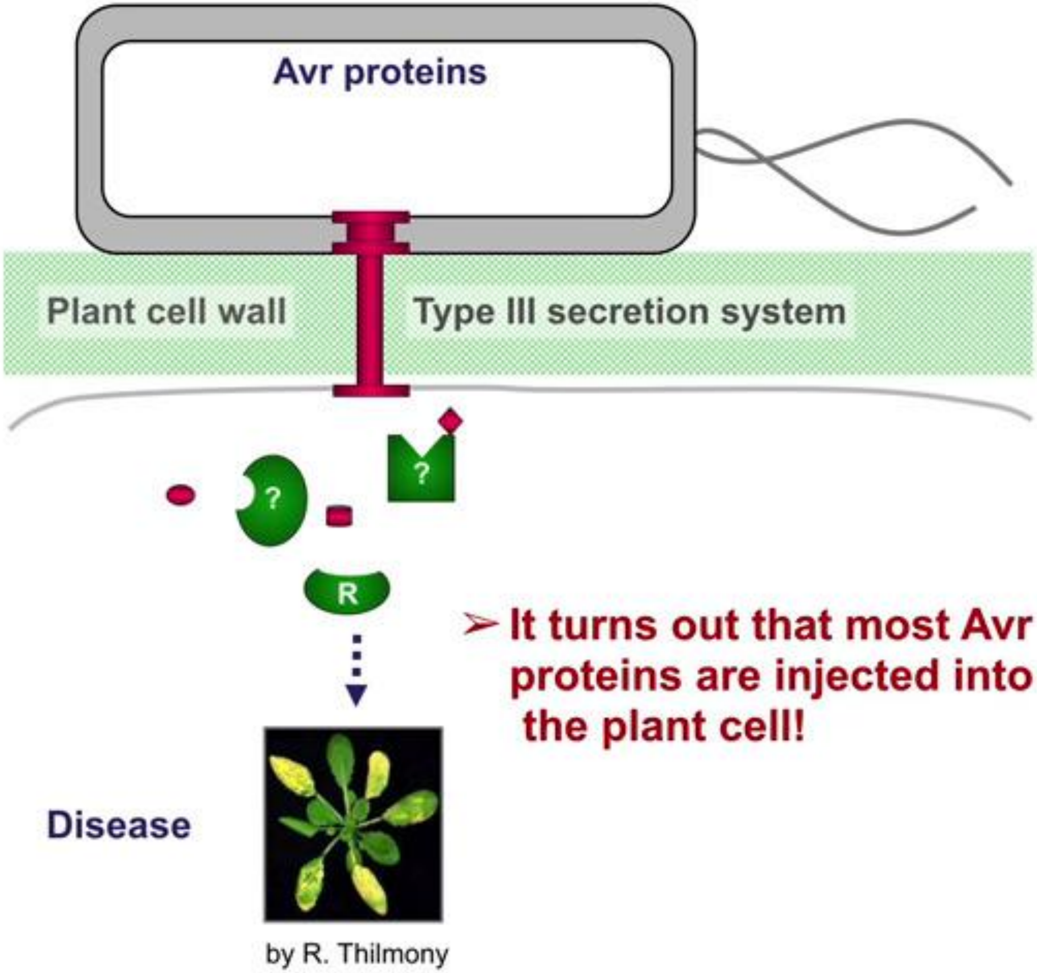
Avr is directly injected into the cells



Avr is directly injected into the cells

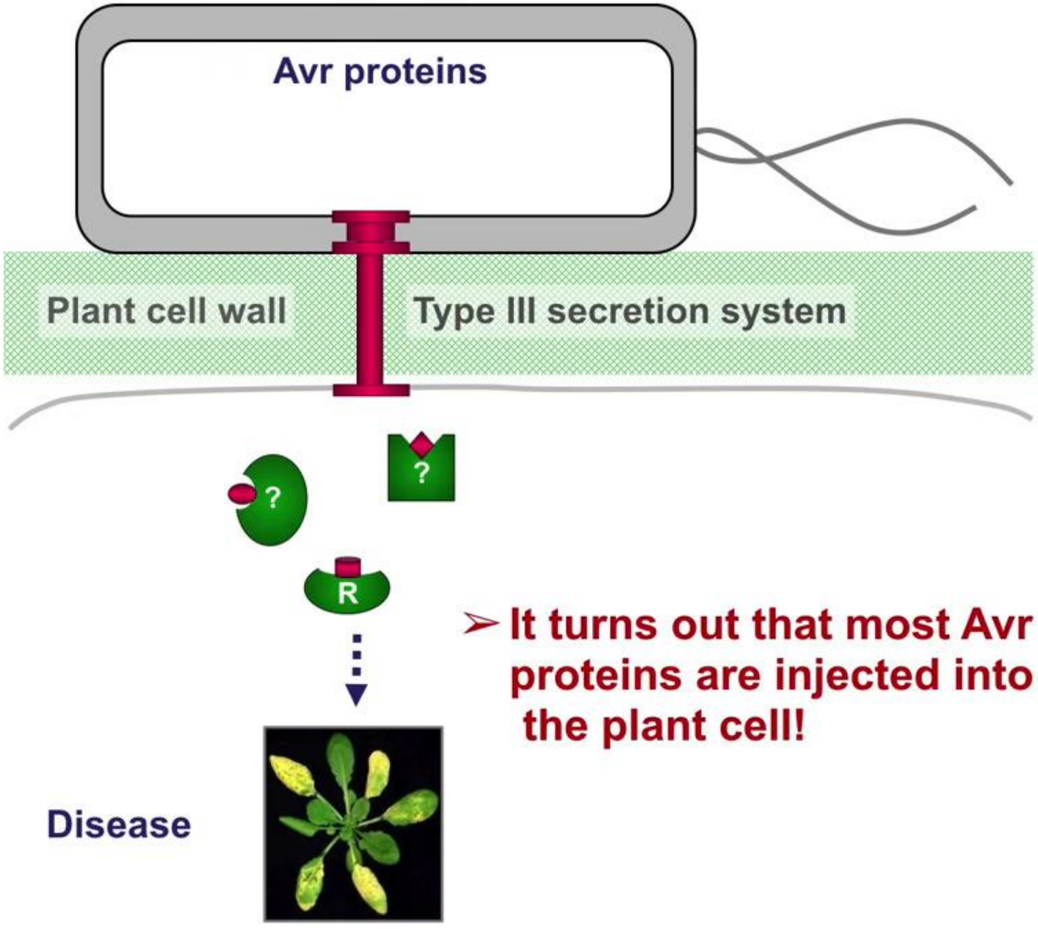


Avr is directly injected into the cells



- It turns out that most Avr proteins are injected into the plant cell!

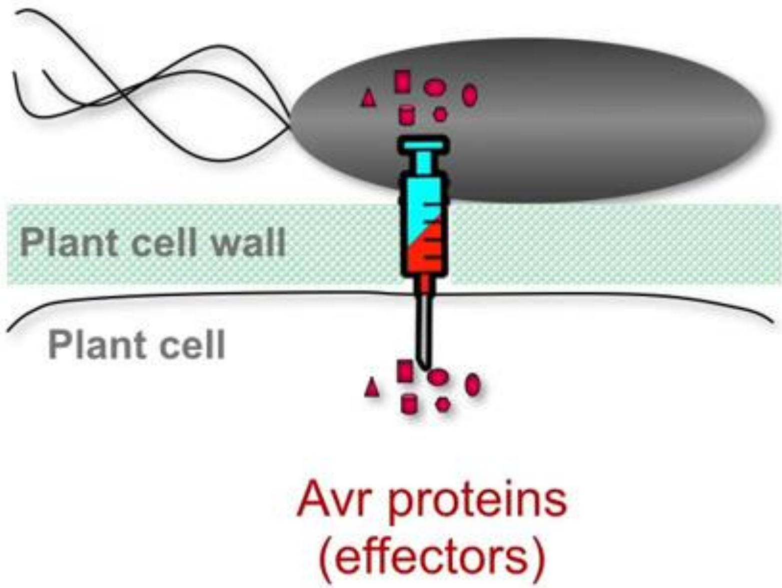
Avr is directly injected into the cells

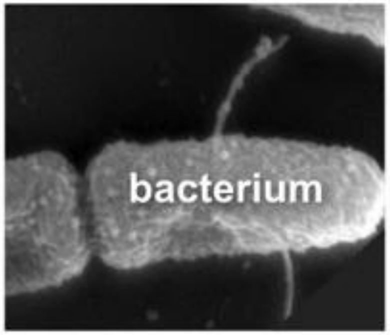


by R. Thilmony

- It turns out that most Avr proteins are injected into the plant cell!

Bacterial type III secretion system

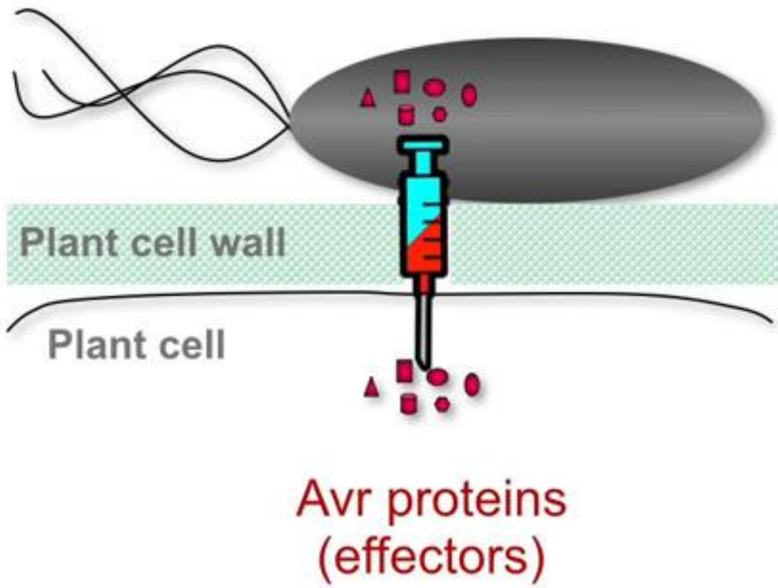


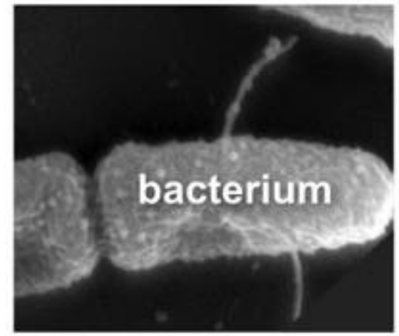


Jin & He (2001) Science

- Conserved in human and plant pathogenic bacteria

Bacterial type III secretion system



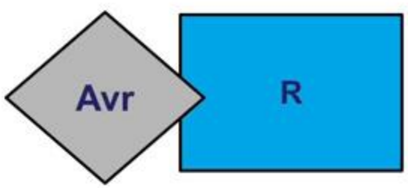


Jin & He (2001) Science

- Conserved in human and plant pathogenic bacteria
- Translocation of effectors also occur in other pathogens (fungi, oomycetes, nematodes)

Plant genomes contain only several hundreds R genes

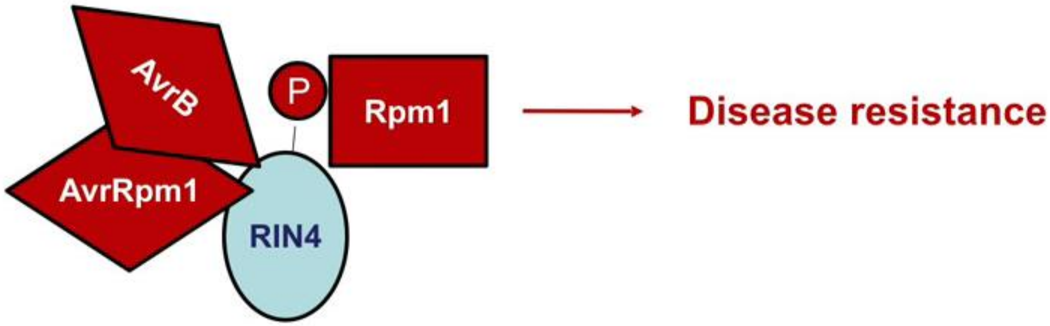
➢ ***How can a limited number of R proteins recognize all pathogens?***



Direct recognition
(in flax rust disease)

Indirect recognition

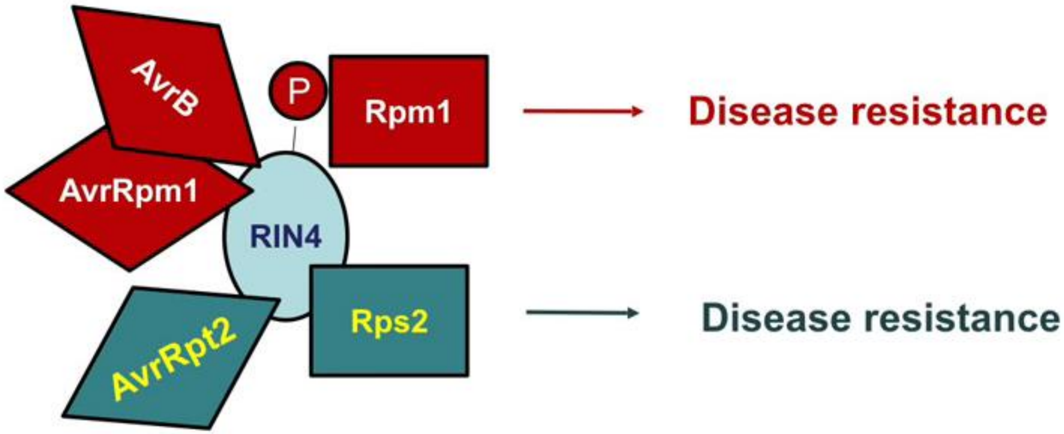
- In many other diseases



- AvrB and AvrRpm1 induces phosphorylation of RIN4

Indirect recognition

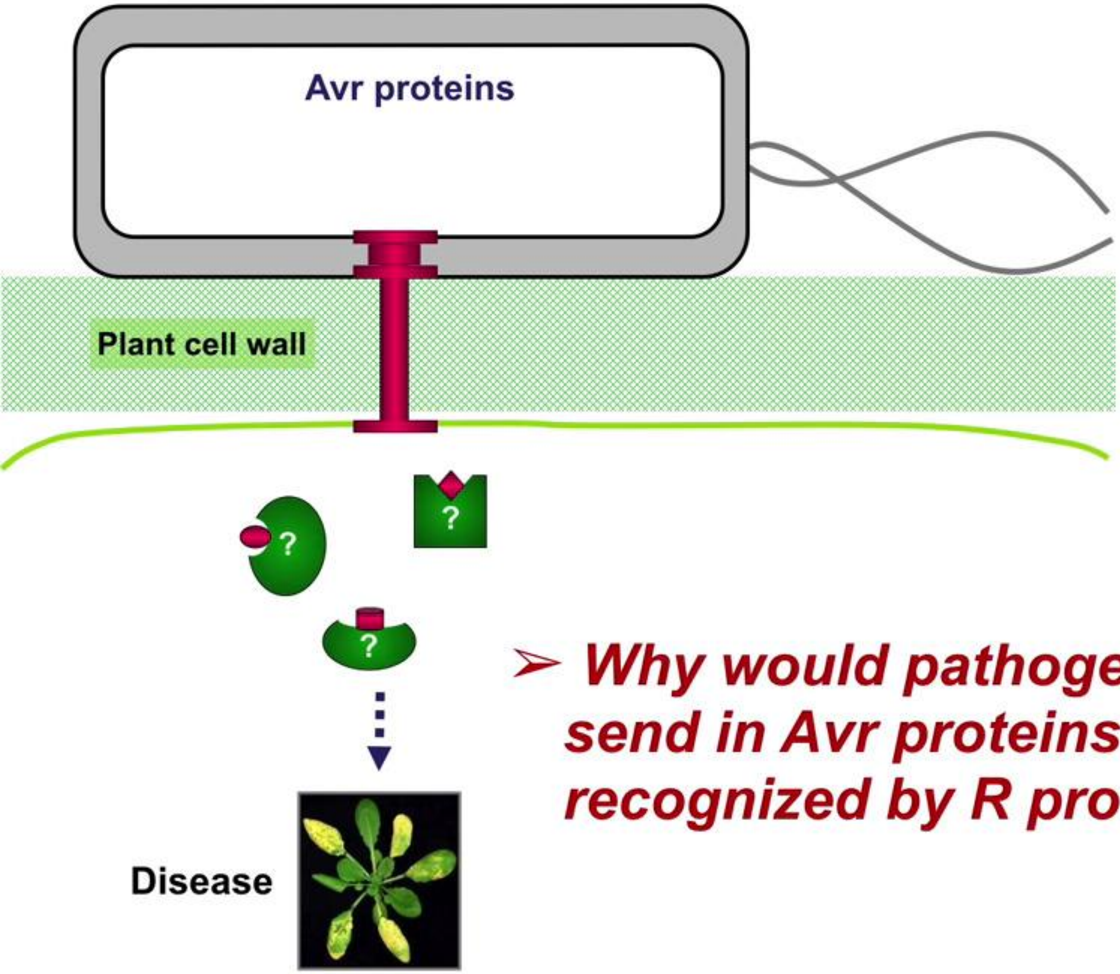
➢ In many other diseases



➢ AvrB and AvrRpm1 induces phosphorylation of RIN4

➢ AvrRpt2 cleaves RIN4

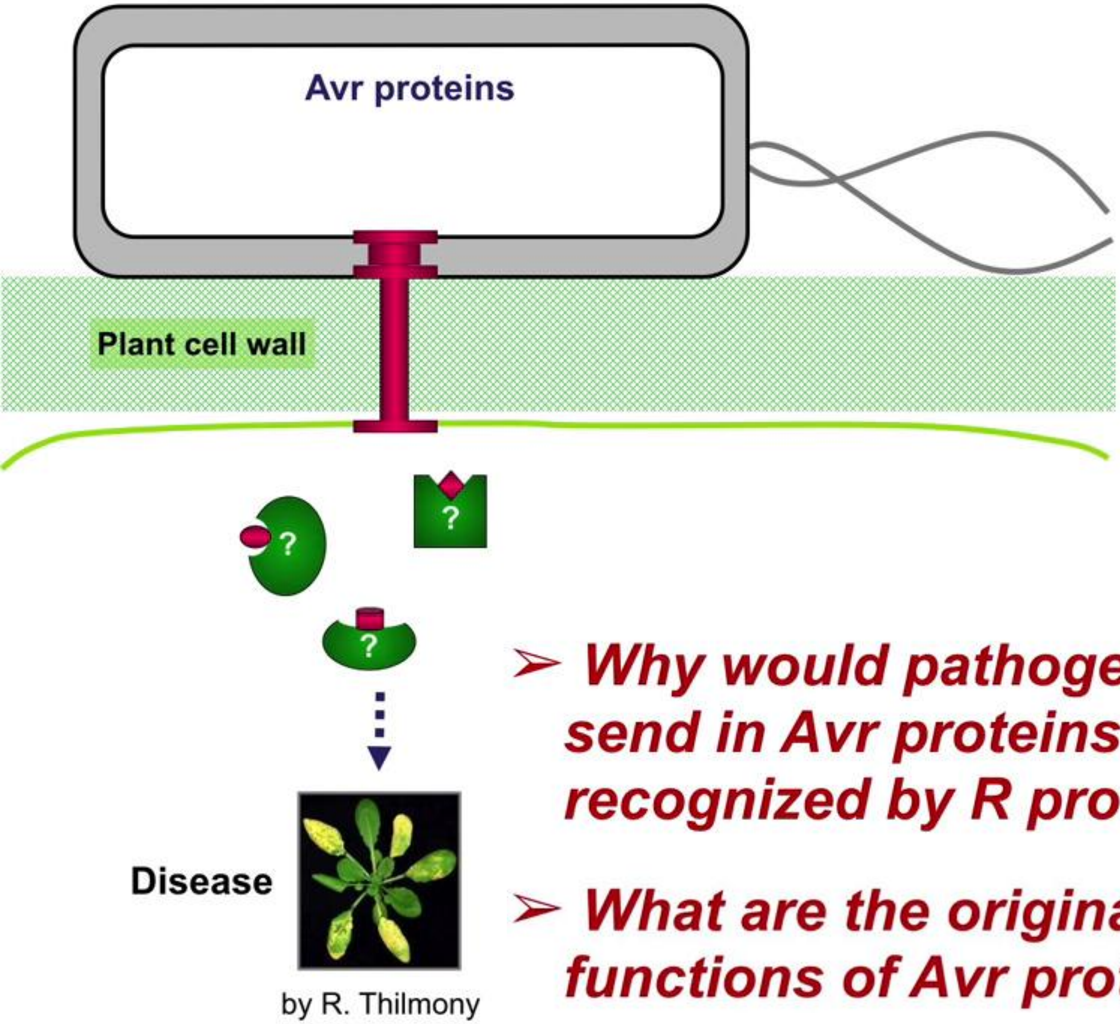
Role of Avr genes



by R. Thilmony

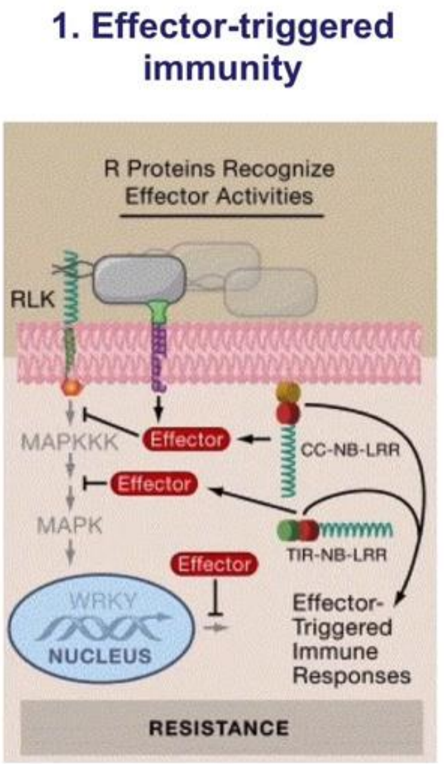
- ***Why would pathogens send in Avr proteins to be recognized by R proteins?***

Avr proteins



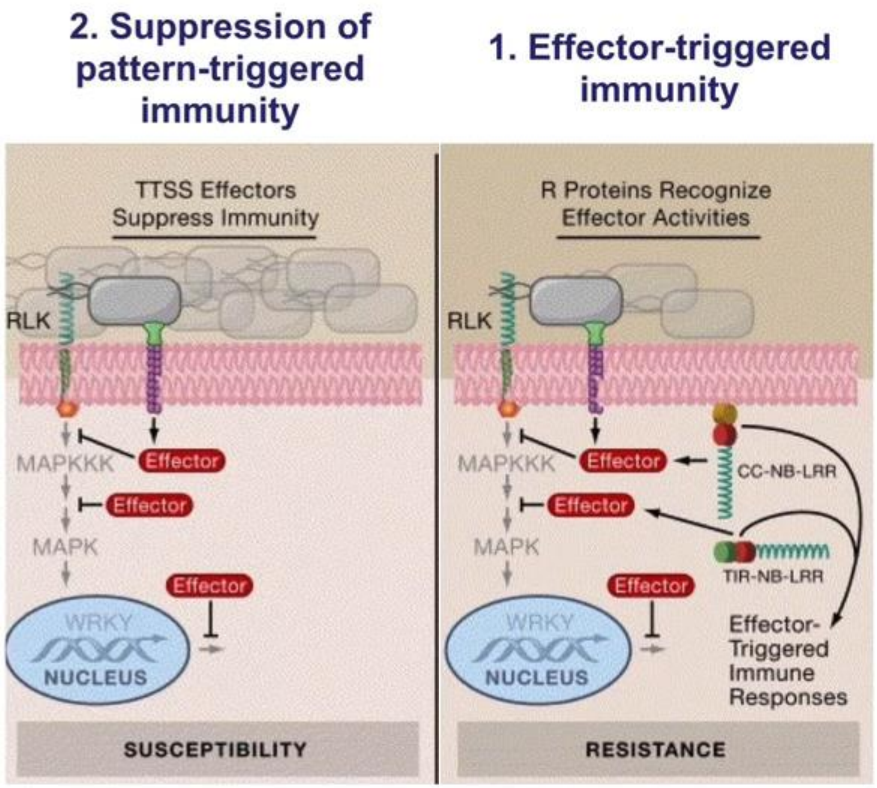
- ***Why would pathogens send in Avr proteins to be recognized by R proteins?***
- ***What are the original functions of Avr proteins?***

Avr attack immunity in absence of R protein



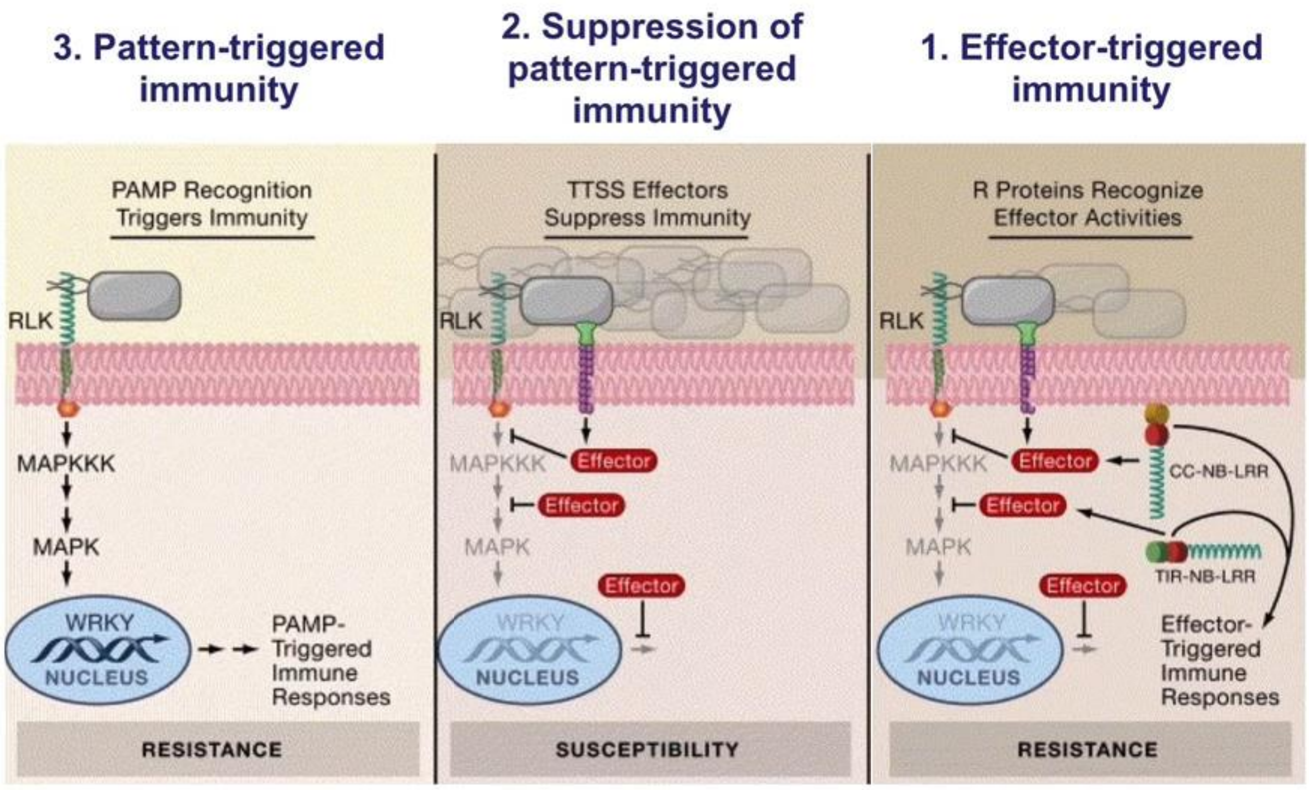
Chisholm et al. (2006) Cell

Avr attack immunity in absence of R protein



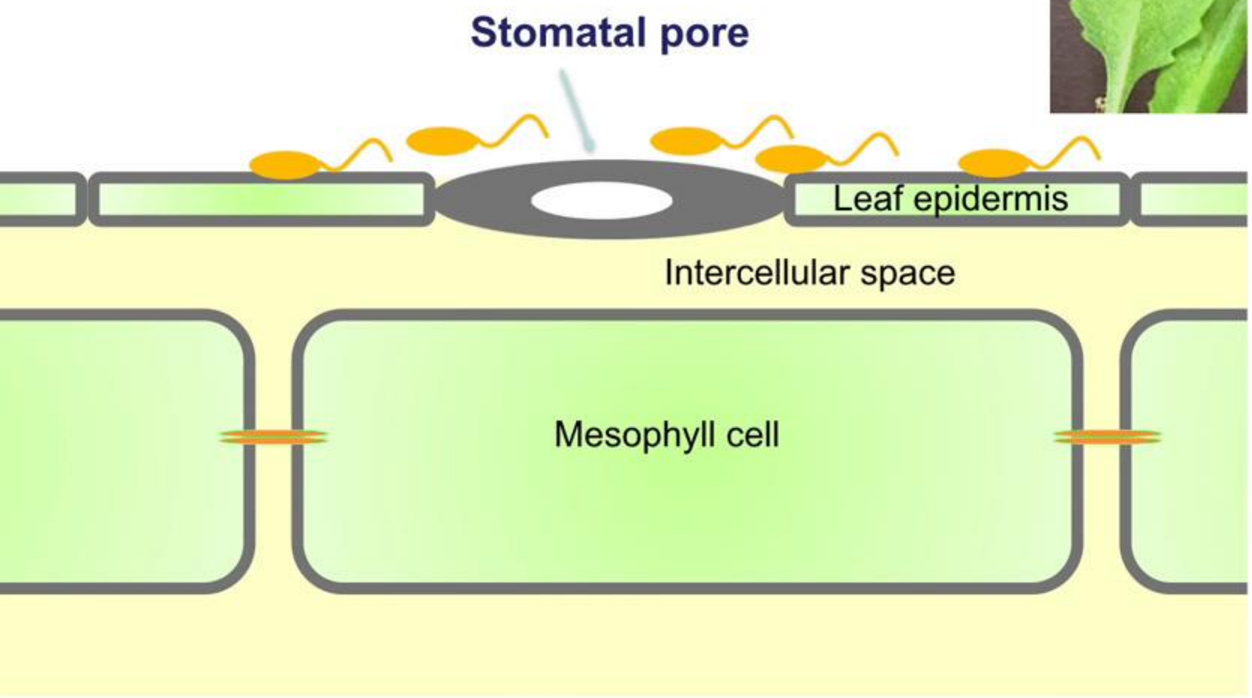
Chisholm et al. (2006) Cell

Avr effectors attack immunity in absence of R protein

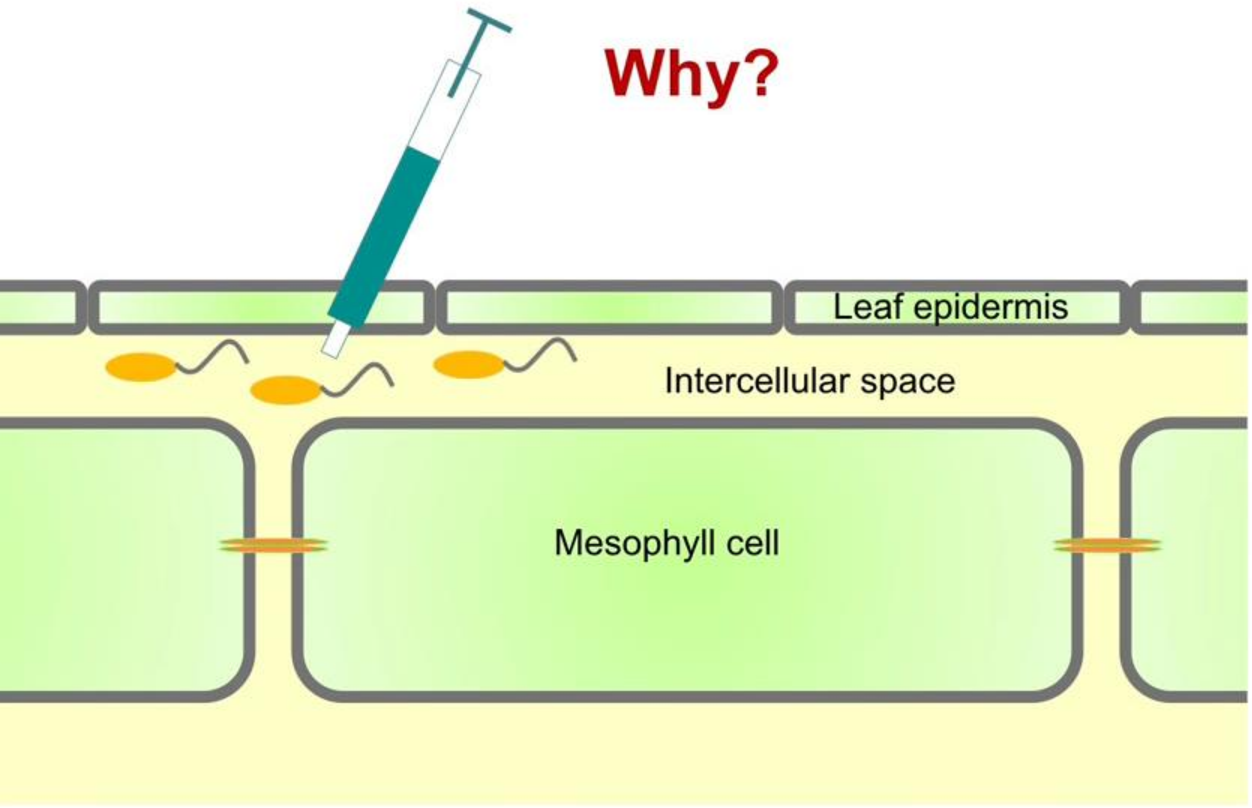


Chisholm et al. (2006) Cell

Bacteria on the plant surface

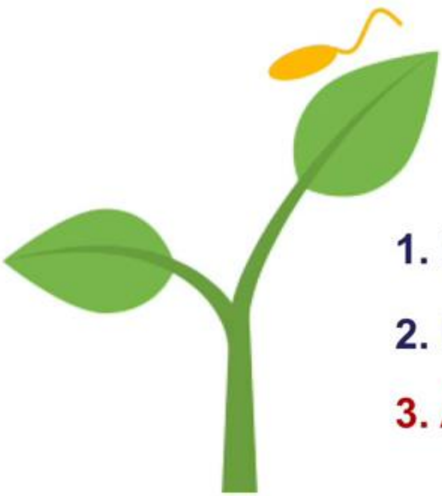


Syringe infiltrated into the leaf



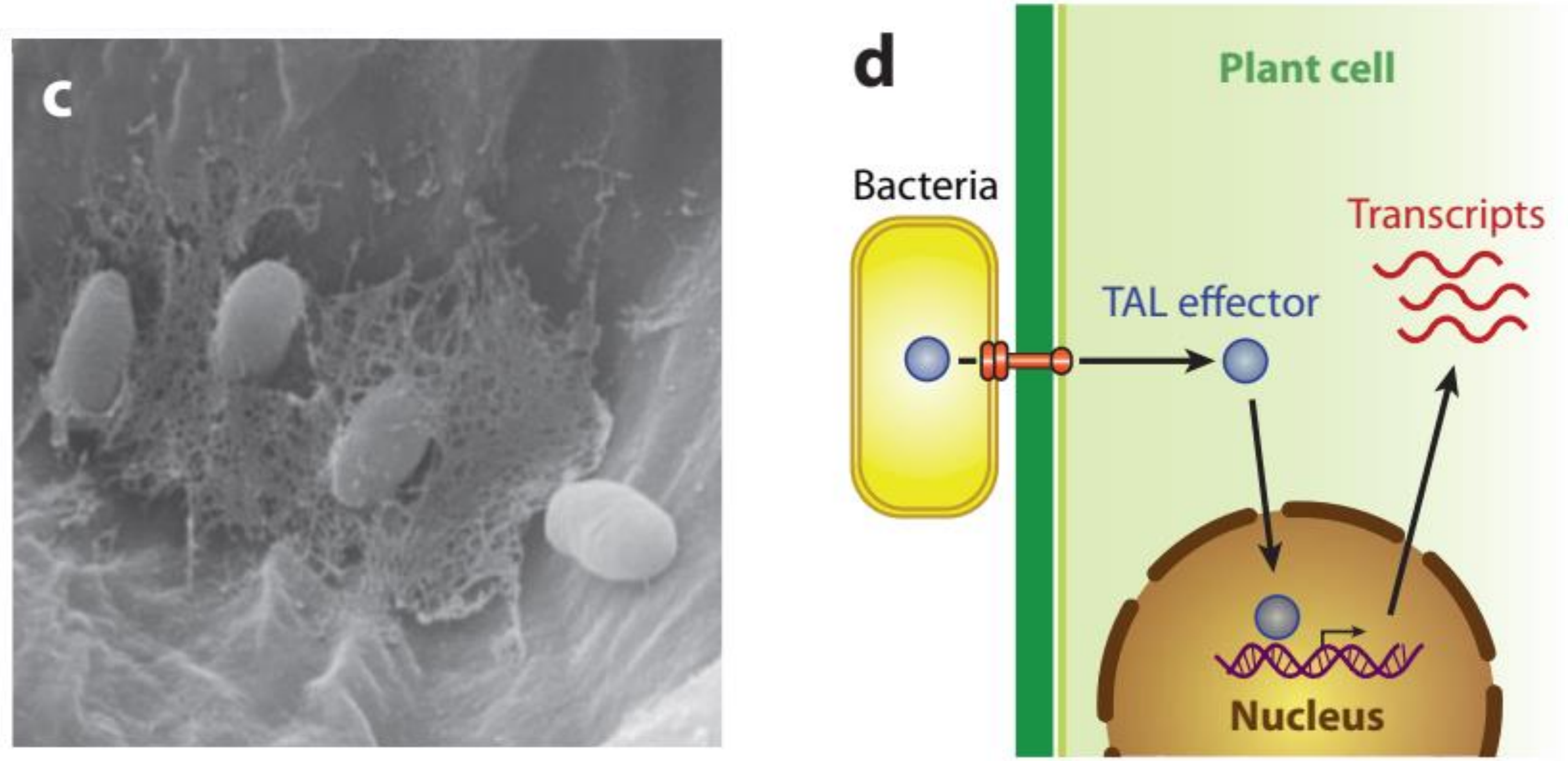
Immune function of stomata

- ➢Two branches of plant innate immune system
- ➢Constantly attacked by pathogen effectors



1. Pattern-triggered immunity
2. Effector-triggered immunity
3. Attacks by pathogen effectors

Transcription activator-like (TAL)



Discovery of TALE

Plant
R gene

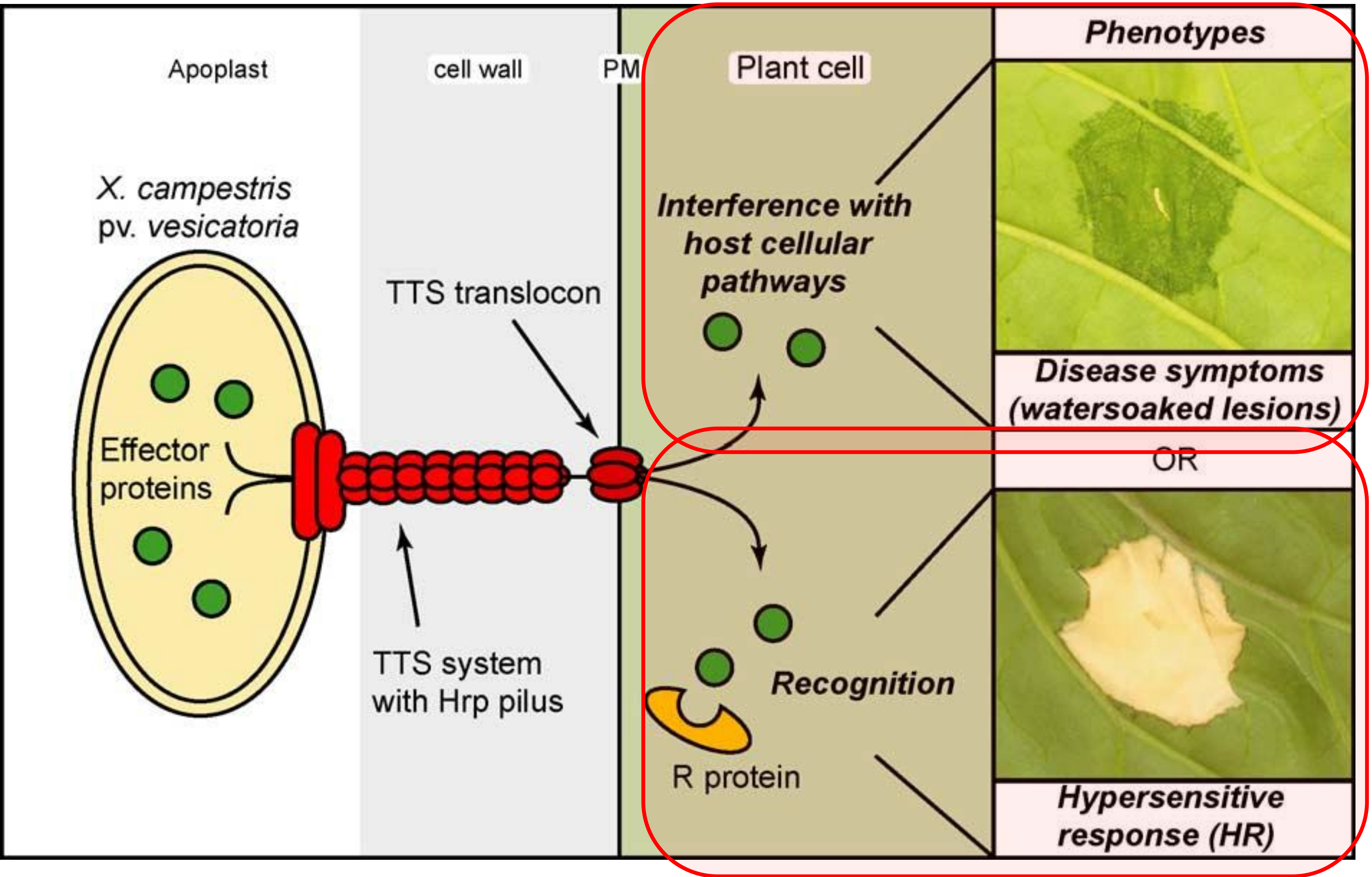
Pathogen
Avr gene

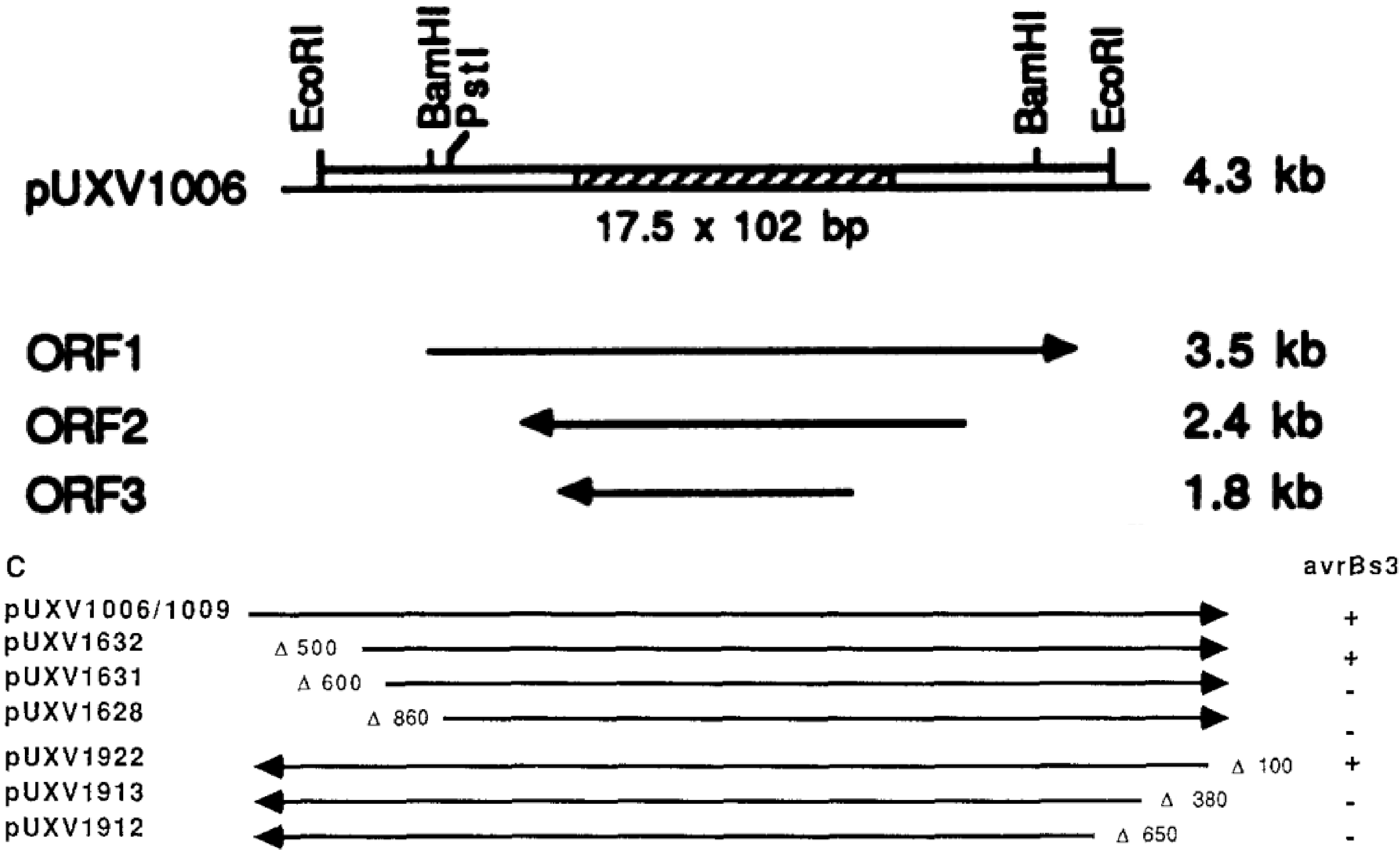
	+ Bs3 ECW-30R	+ Bs1 ECW-10R
+ AvrBs3	R: resistance	S: susceptible
- AvrBs3	S	S

Robert stall and team

University of Florida

Discovery of TAL-Effectors

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BamHI
PstI
BamHI
EcoRI
pUXV1006
4.3 kb
17.5 x 102 bp
ORF1
3.5 kb
ORF2
2.4 kb
ORF3
1.8 kb
C
avrBs3
pUXV1006/1009
+
pUXV1632
Δ 500
+
pUXV1631
Δ 600
-
pUXV1628
Δ 860
-
pUXV1922
Δ 100
+
pUXV1913
Δ 380
-
pUXV1912
Δ 650
-

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Q   Q   Q   Q   E   K   I   K   P   K   V   R   S   T   V   A   Q   H   H   E   A   L   V   G   H   G   F   T   H   A   H   I   V   A
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GTCGTCGTTGTCCTCTTCTAGTTTGGCTTCCAAGCAAGCTGTCACCGCGTCGTGGTGCTCCGTGACCAGCCGGTACCCAAATGTGTGCGCGTGTAGCAAC

  L   S   Q   H   P   A   A   L   G   T   V   A   V   K   Y   Q   D   M   I   A   A   L   P   E   A   T   H   E   A   I   V   G   V
CGCTCAGCCAACACCCGGCAGCGTTAGGGACCGTCGCTGTCAAGTATCAGGACATGATCGCAGCGTTGCCAGAGGCGACACACGAAGCGATCGTTGGCGT 1300
GCGAGTCGGTTGTGGGCCGTCGCAATCCCTGGCAGCGACAGTTCATAGTCCTGTACTAGCGTCGCAACGGTCTCCGCTGTGTGCTTCGCTAGCAACCGCA
                                                                    *   S   M   I   A   A   N   G   S   A   V   C   S   A   I   T   P   T

 G   K   Q   W   S   G   A   R   A   L   E   A   L   L   T   V   A   G   E   L   R   G   P   P   L   Q   L   D   T   G   Q   L   L
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  P   L   C   H   D   P   A   R   A   R   S   A   K   S   V   T   A   P   S   N   L   P   G   G   N   C   N   S   V   P   W   S   R

K   I   A   K   R   G   G   V   T   A   V   E   A   V   H   A   W   R   N   A   L   T   G   A   P   L   N   L   T   P   E   Q   V   V
AAGATTGCAAAACGTGGCGGCGTGACCGCAGTGGAGGCAGTGCATGCATGGCGCAATGCACTGACGGGTGCCCCCCTGAACCTGACCCCGGAGCAGGTGG 1500
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 L   I   A   F   R   P   P   T   V   A   T   S   A   T   C   A   H   R   L   A   S   V   P   A   G   R   F   R   V   G   S   C   T

  A   I   A   S   H   D   G   G   K   Q   A   L   E   T   V   Q   R   L   L   P   V   L   C   Q   A   H   G   L   T   P   Q   Q   V
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T   A   M   A   L   W   S   P   P   L   C   A   S   S   V   T   C   R   S   N   G   T   S   H   W   A   W   P   R   V   G   C   C   T

 V   A   I   A   S   N   G   G   G   K   Q   A   L   E   T   V   Q   R   L   L   P   V   L   C   Q   A   H   G   L   T   P   Q   Q
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  T   A   M   A   L   L   P   P   P   L   C   A   S   S   V   T   C   R   S   N   G   T   S   H   W   A   W   P   R   V   G   C   C

V   V   A   I   A   S   N   S   G   G   K   Q   A   L   E   T   V   Q   R   L   L   P   V   L   C   Q   A   H   G   L   T   P   E   Q
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  V   V   A   I   A   S   N   G   G   G   K   Q   A   L   E   T   V   Q   R   L   L   P   V   L   C   Q   A   H   G   L   T   P   E
AGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCCCGGA 1900
TCCACCACCGGTAGCGGTCGTTACCGCCACCGTTCGTCCGCGACCTCTGCCACGTCGCCGACAACGGCCACGACACGGTCCGGGTACCGGACTGGGGCCT
C   T   T   A   M   A   L   L   P   P   P   L   C   A   S   S   V   T   C   R   S   N   G   T   S   H   W   A   W   P   R   V   G   S

 Q   V   V   A   I   A   S   N   I   G   G   K   Q   A   L   E   T   V   Q   A   L   L   P   V   L   C   Q   A   H   G   L   T   P
GCAGGTGGTGGCCATCGCCAGCAATATTGGTGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCCCG 2000
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  C   T   T   A   M   A   L   L   I   P   P   L   C   A   S   S   V   T   C   A   S   N   G   T   S   H   W   A   W   P   R   V   G

E   Q   V   V   A   I   A   S   N   I   G   G   K   Q   A   L   E   T   V   Q   A   L   L   P   V   L   C   Q   A   H   G   L   T   P
GAGCAGGTGGTGGCCATCGCCAGCAATATTGGTGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCC 2100
CTCGTCCACCACCGGTAGCGGTCGTTATAACCACCGTTCGTCCGCGACCTCTGCCACGTCCGCGACAACGGCCACGACACGGTCCGGGTACCGGACTGGG
 S   C   T   T   A   M   A   L   L   I   P   P   L   C   A   S   S   V   T   C   A   S   N   G   T   S   H   W   A   W   P   R   V
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Fig. 3

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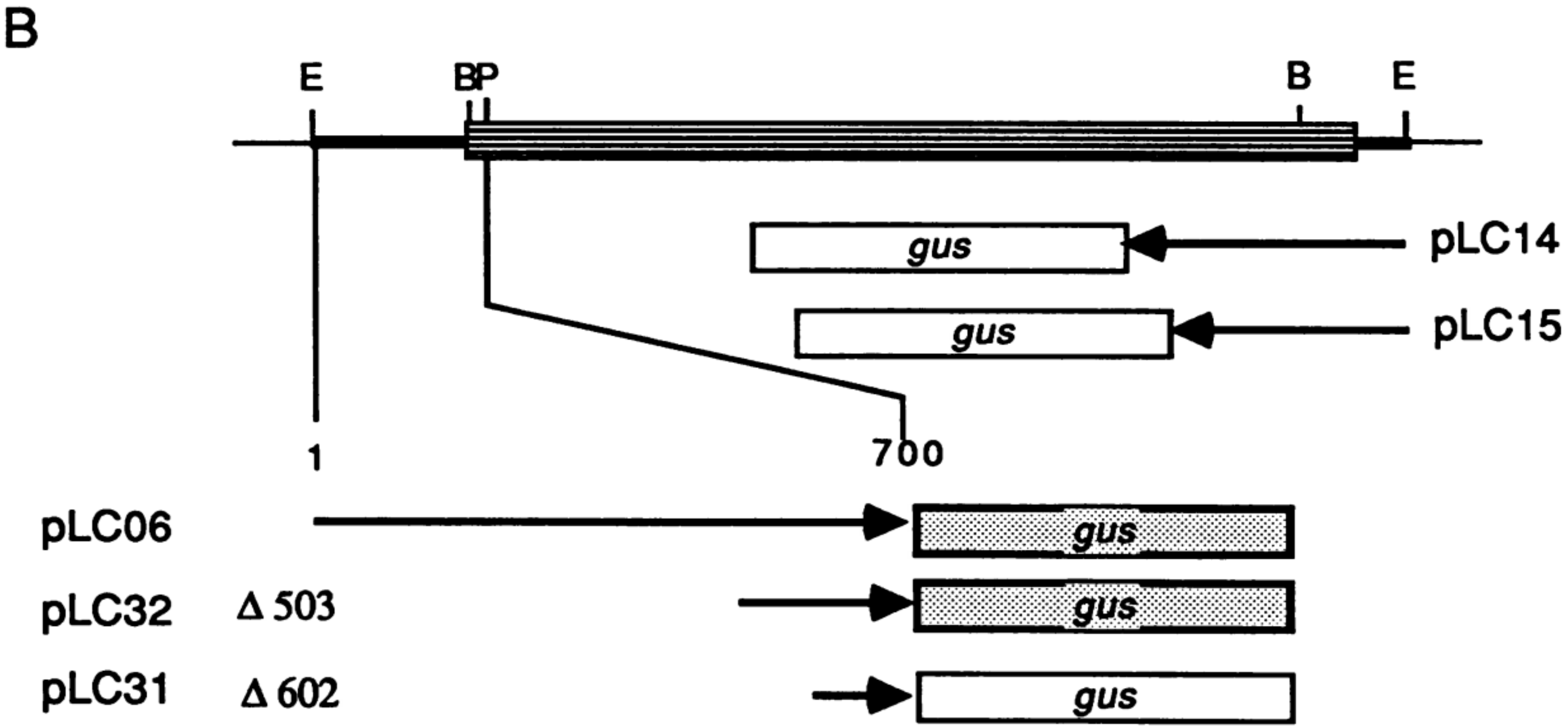
              1                                                                                                    102
rep  1        CTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCCACGATGGCGGCAAGCAGGCGCTGGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGCCCCATGGC
rep  2        .........C............................C..T............................................................
rep  3        .........C..........................A.C..T............................................................
rep  4        ......................................C..T............................................................
rep  5        ....................................AT...T...........................GC...............................
rep  6        ....................................AT...T...........................GC...............................
rep  7        ....................................AT...T...........................GC...............................
rep  8        .................................C.C.A...C............................................................
rep  9        .................................C.C.A...C............................................................
rep 10        .........C............................C..T............................................................
rep 11        ....................................A.C..T...........................GC...............................
rep 12        ....................................A.C..T............................................................
rep 13        .................................C.C.A...C............................................................
rep 14        .................................C.C.A...C............................................................
rep 15        .................................C.C.A...C............................................................
rep 16        .........C............................C..C....G..C....................................................
rep 17        .................................C.C.A...C............................................................
rep 18        .........C............................C..C....G..C...........GCA.TGTTGCC.A...AT.TCGC.CTGAT.C...GTTG.C.

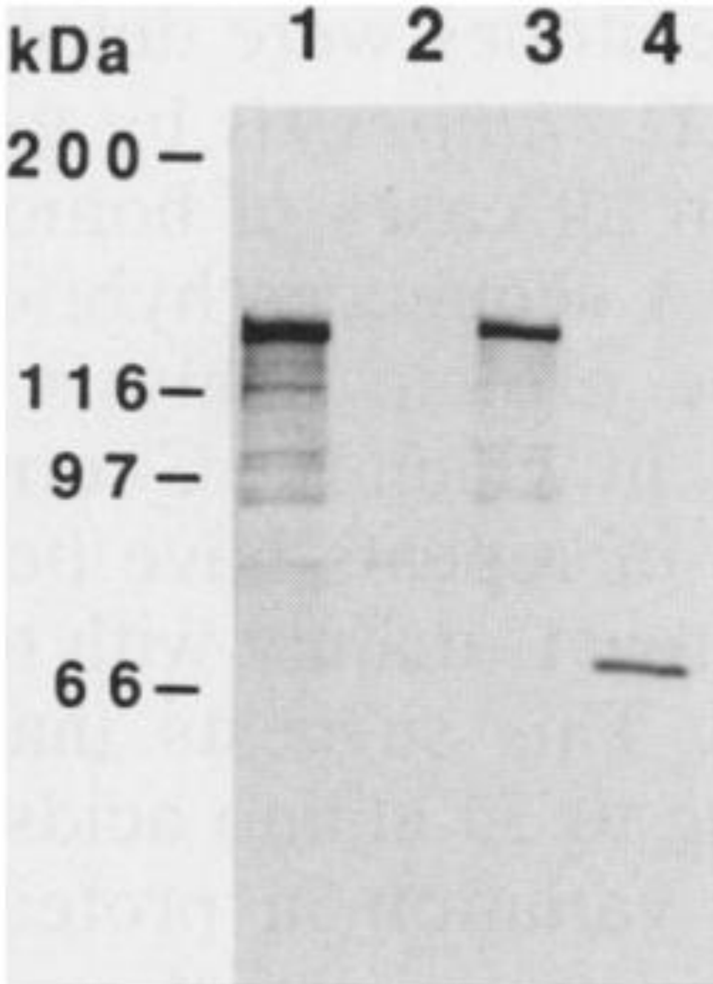
consensus     CTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATGGTGGYGGCAAGCAGGCGCTGGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGC

aa            LeuThrProGluGlnValValAlaIleAlaSerHisAspGlyGlyLysGlnAlaLeuGluThrValGlnArgLeuLeuProValLeuCysGlnAlaHisGly
                       Gln                       AsnGly      ArgPro                  Ala
                                                    Ser
                                                    Ile
```

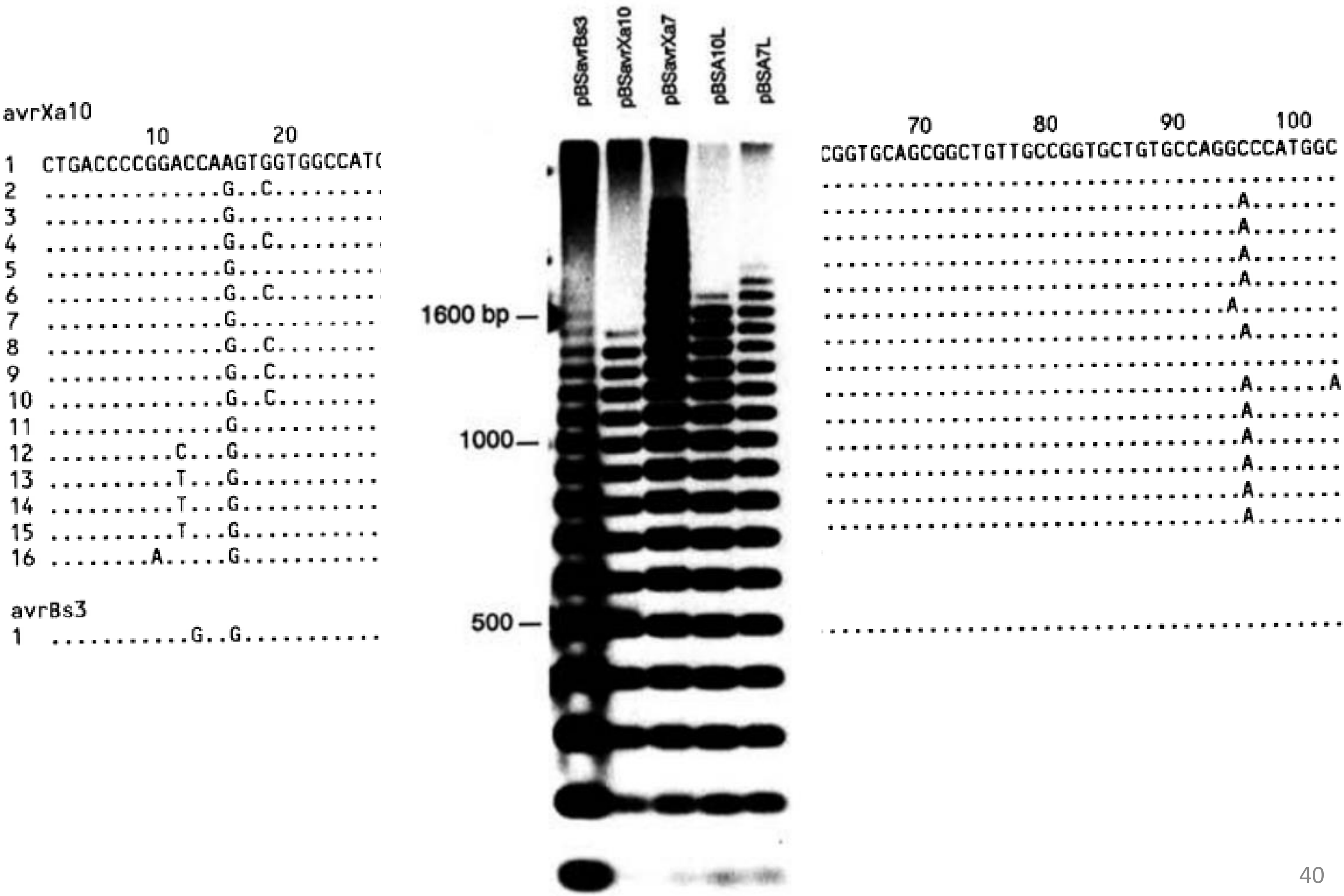
Expression Studies



Expression Studies



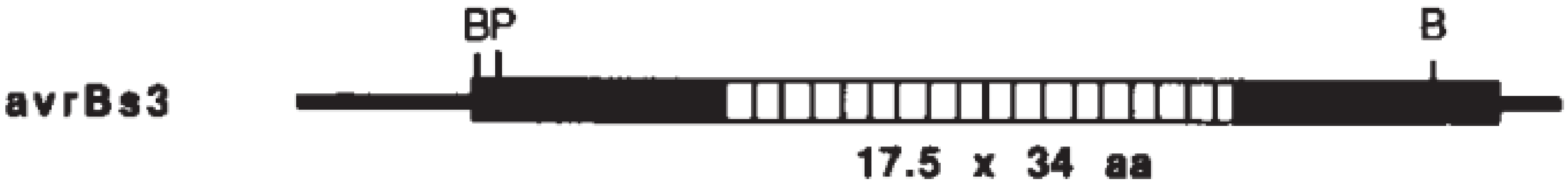
Isolation of AvrBs3 Homologs



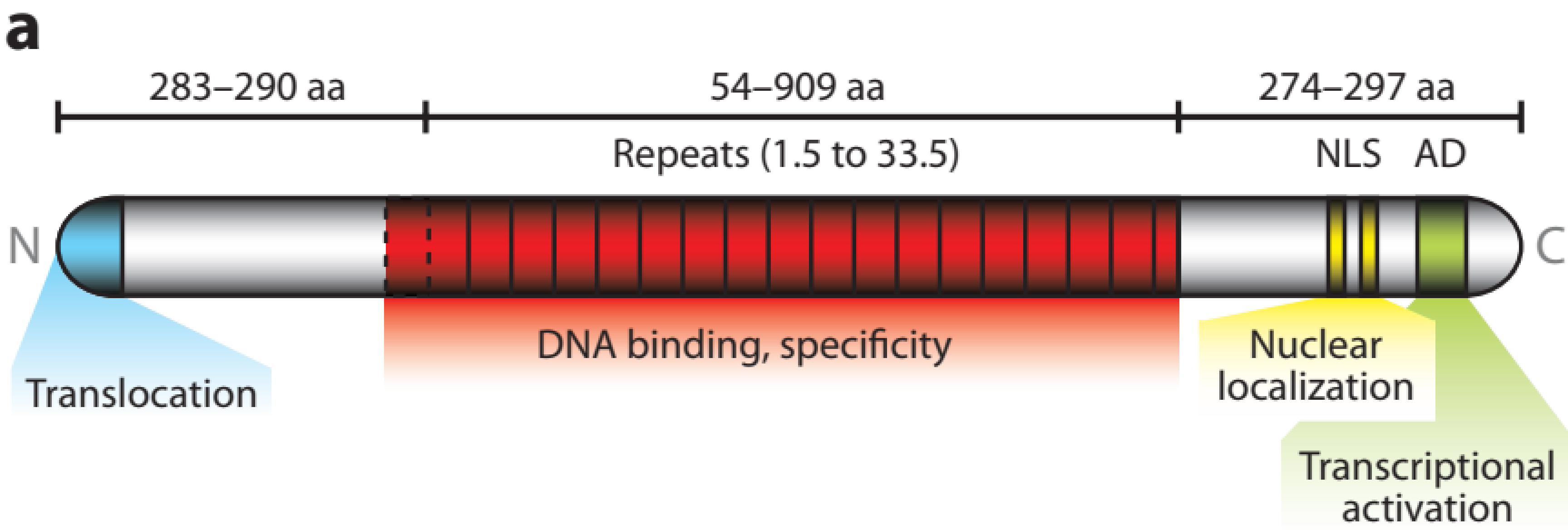
```
               VARIABLE
                REGION
avrXa10
1   LTPDQVVAIASNIGGNQALETVQRLLPVLCQAHG
2   ...........HG..K..................
3   ........S..NI..K...A...........D..
4   ...........HG..K...............D..
5   ...........NI..K...............D..
6   ...........NI..K...............D..
7   ..........NNN..K...............T..
8   ...........HD..K...............D..
9   ...........NI..K...A...........A..
10  ...........HD..K............V..D..
11  ..........NNN..K...............D..
12  ...A.......HG..K...............D..
13  ...V.......NS..K...............D..
14  ...V.......NG..K...A...........D..
15  ...V.......HD..K...............D..
16  ...........NG..K...

avrBs3
1   ...E.......HD..K...............A..
```

The repeats Determine the Specificity of Action



The repeats Determine the Specificity of Action



Conclusions of Lecture-13

- Xanthomonas secretes effectors through T3S system.
- Many Type3 effectors = TALE = largest family = transcriptional activators of plant genes.
- Gene to gene relationship = avrBs3 isolation & characterization.
- Homologs = avrXa10 and others = HVR = 12 & 13th
- Mutational & Deletion studies = Repeats order

Questions??