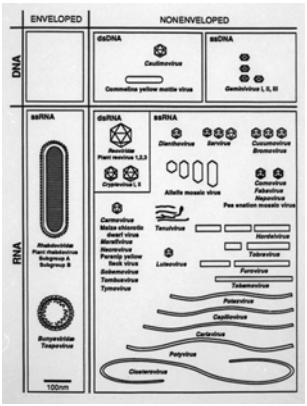
Plant viruses are a highly diverse group of pathogens.

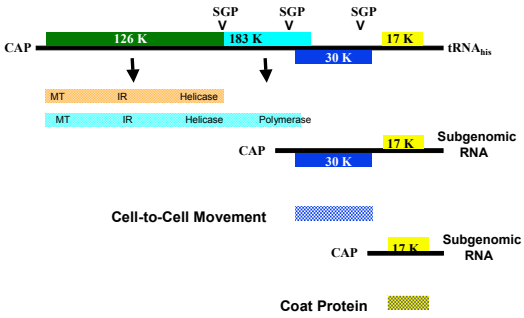
Many show a high degree of similarity with animal viruses.

Plant viruses have evolved unique genes/functions to facilitate plant infection.

- Movement
- Vector transmission

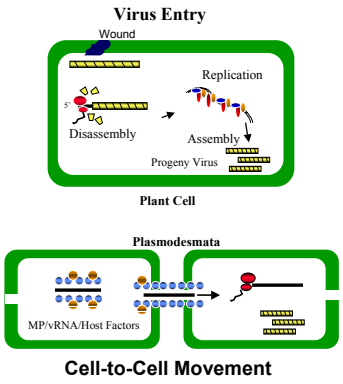


TMV Genome Organization

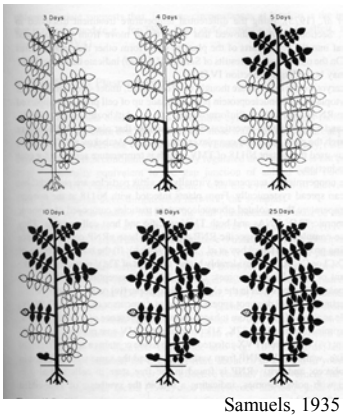


Protein Expression Chart
Synchronous Infections
Magic Box

Virus Life Cycle

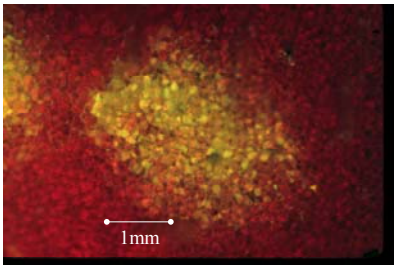


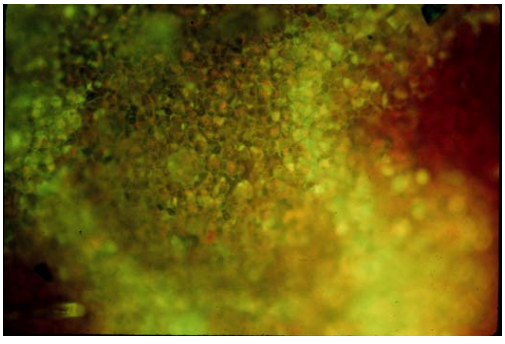
Systemic Movement



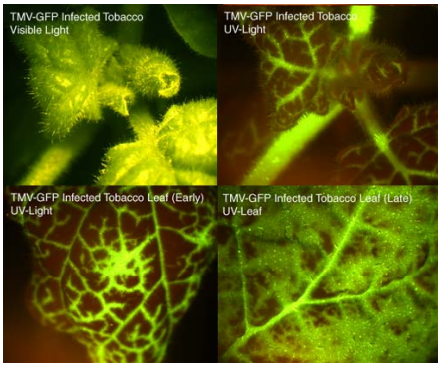
Samuels, 1935

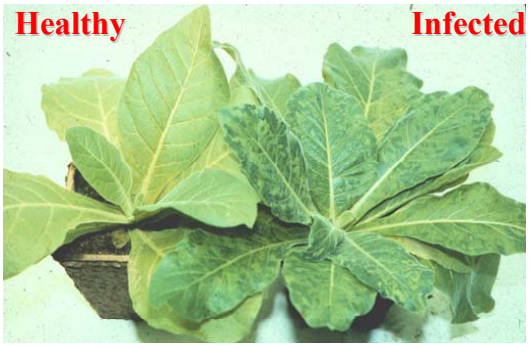
Single TMV-GFP infection site on an inoculated leaf of tobacco.
(Four days post-inoculation)



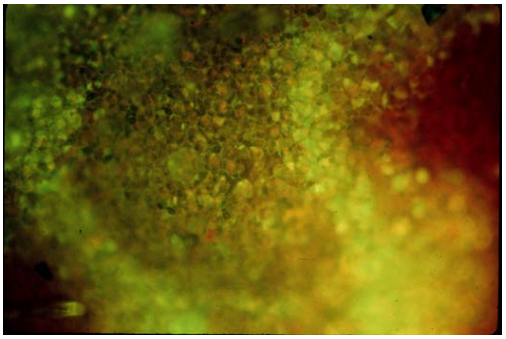


Replication at leading edge
Plasmodesmata gating at leading edge





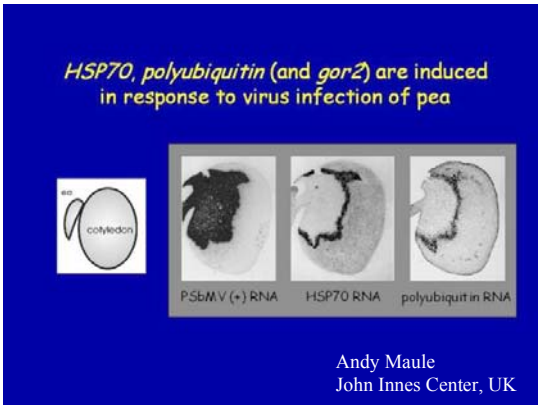
Microarray Data From TMV Infected Whole Leaf Tissues



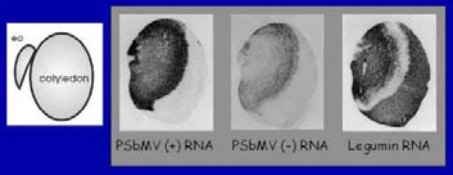
Transcriptional Alterations During Virus Replication

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

Sheetal Golem, 2003

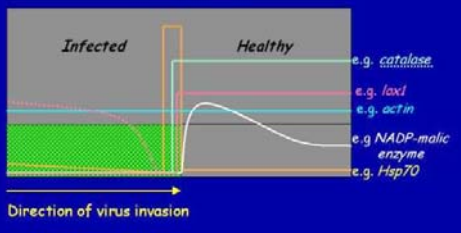


PSbMV replication is associated with an inhibition of host gene expression



Andy Maule

Changes in host gene expression in response to PSbMV/CMV infection



Andy Maule

Virus-Host Interactions

1. Essential for virus function
   - Replication
   - Movement
2. Host Resistance Interactions
3. Inconsequential to virus function

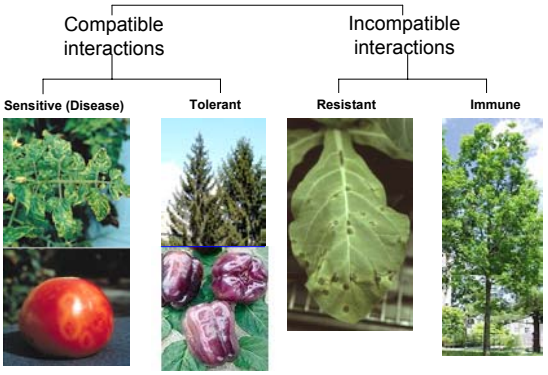
Virus → Host

Disturbance in the host physiology

Proteome
Transcriptome
Metabolic Pathways etc. → **Disease/Resistance**
Chlorosis, Necrosis
Growth abnormalities

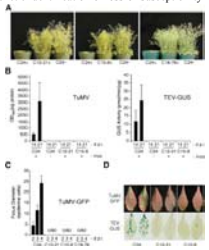
Plant Cell

Virus - Host Responses

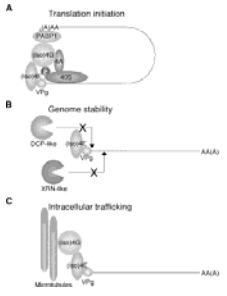


Immune Response: Essential Role for eIF(iso)4E in Potyvirus Infection

Genetic identification of loss-of-susceptibility mutants



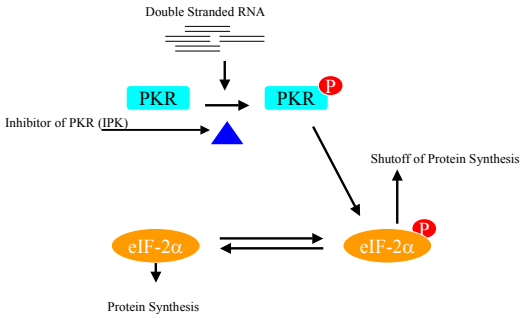
Mechanisms for immune response



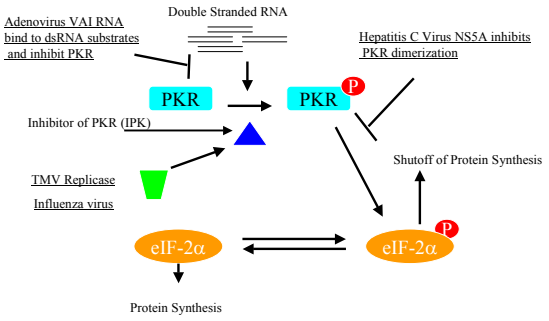
Lellis et al., 2002 Current Biology

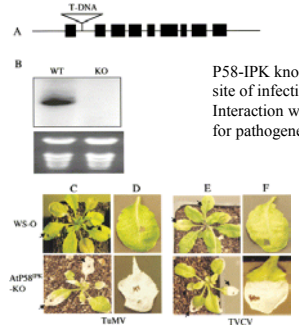
Disease Resistance / Avoidance

Inhibitor of Protein Kinase R



Inhibitor of Protein Kinase R

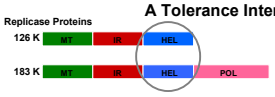


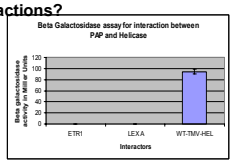


P58-IPK knockout results in necrosis at the site of infection.
Interaction w/viral protein important for pathogenesis.

Bilgin et al., 2003, Developmental Cell

HELICASE INTERACTION WITH PAP1/IAA26: A Tolerance Interactions?





Helicase domain showed a strong interaction with Phytochrome Associated Protein(PAP1) or IAA26.

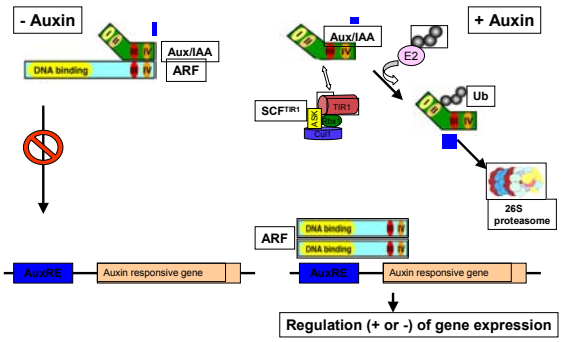
HEL

IAA26 is an auxin responsive regulatory protein and a member of the Aux/IAA family of transcription factors

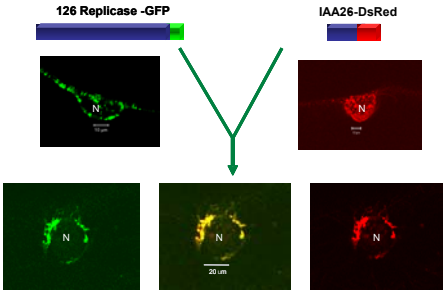
Reed J.W, Trends Plant Sci. 2001 6(9)

ARF's - transcriptional factors that bind to Auxin responsive Elements (AuxRE) found in the promoters of early auxin responsive genes.

MODEL FOR THE FUNCTION OF ARF AND Aux/IAA PROTEINS



IAA26-DSRED IS HELD OUT OF THE NUCLEUS BY TMV-126 kDa REPLICASE-GFP



TRANSCRIPTIONALLY ALTERED ARABIDOPSIS GENES CONTAIN AUXIN RESPONSIVE ELEMENTS WITHIN THEIR PROMOTERS

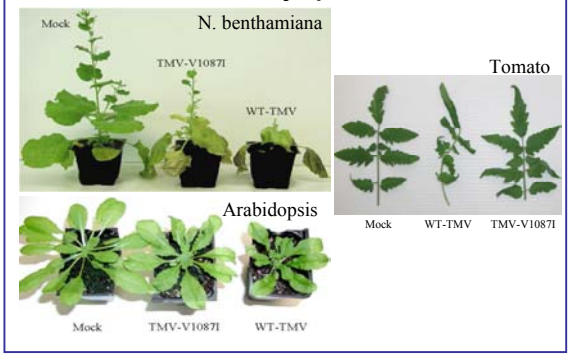
- **Microarray data identified 68 Arabidopsis genes altered in infected tissue (Golem et al. 2003)**
- **Promoter analysis of 2 kb region upstream of each gene to identify Aux RE's TGTCTC element – ARF binding site**
- **20 genes contained 2 or more AuxRE's. ~ 30% of the genes displaying transcriptional alterations to TMV infection may be linked to auxin response system**

AFGC Gene Model ID	Protein name	Number of AuxRE's	Fold expression: In TMV infection (from microarray)	Fold expression: +50μM IAA (qRT-PCR derived)
At5g02160	Put protein	3	-1.9	-4.09
At1g19350	Unk protein	3	-2.0	-1.8
At3g17790	Acid Phos'tase type 5	2	-2.3	-6.6
At5g21010	Put stress prot	3	-3.5	-1.5
At4g38850	SAUR-AC1	1	-1.10	5.08

+ Not above 95%confidence interval cutoff

- **Auxin induced expression trends similar to that seen in TMV infection**

TMV V1087I infection results in attenuated disease symptoms.



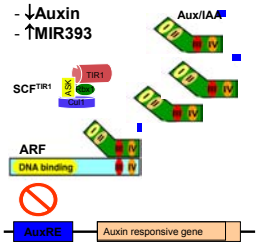
?REPLICASE - AUX/IAA INTERACTION ALTERS CELL ACTIVITY?

Older/Stressed Tissue

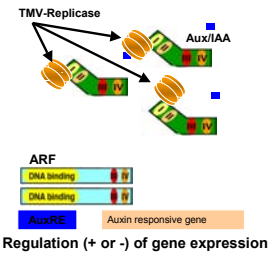
- -Lower Concentration of Auxin
- -Lower Levels of TIR1 due to Increased Accumulations of the Stress-Inducible MicroRNA MIR393 (Sunkar and Zhu, 2004)
- -Increased Levels of AUX/IAA Proteins.

Developmentally Static: Less Suitable for Virus Replication

- ↓Auxin
- ↑MIR393



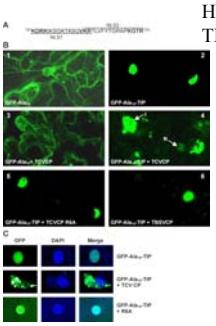
Developmentally Active: More Suitable for Virus Replication

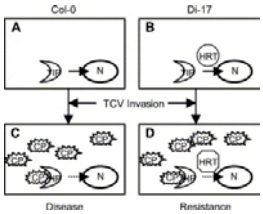


Regulation (+ or -) of gene expression

Hypersensitive Resistance Against Turnip Crinkle Virus

HRT = R gene protein (NBS-LRR)
TIF = NAC family transcription factor

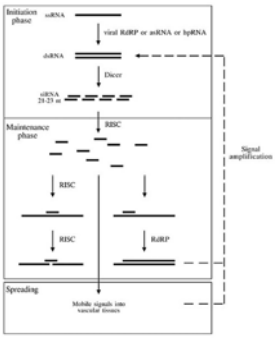




Ren et al., 2005, Virology

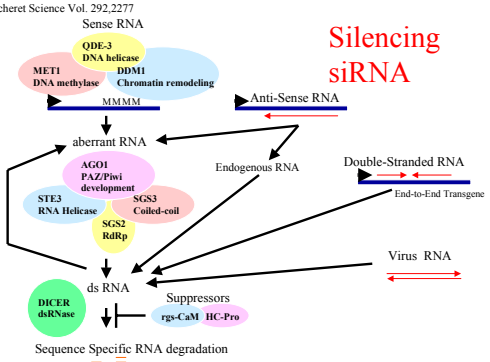
D. Gonsalves, Cornell University

RNAi (PTGS, RNA suppression, VIGS)



Micro RNA (miRNA) = Transcribed directly from the genome and used to regulate various genes.
Short Interfering RNA (siRNA) = originate from viruses, mRNAs, transposons, transgenes

Vance & Vaucheret Science Vol. 292,2277



Sequence Specific RNA degradation

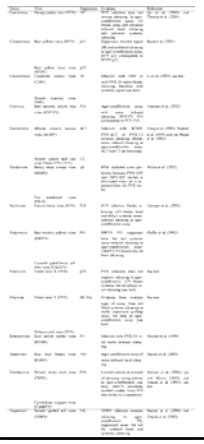
22-28 nucleotide RNAs

Short Interfering RNA (siRNA) = originate from viruses, mRNAs, transposons, heterochromatic DNA, transgenes

Viral suppressors of RNAi

Diverse group of viral proteins sharing no common sequences

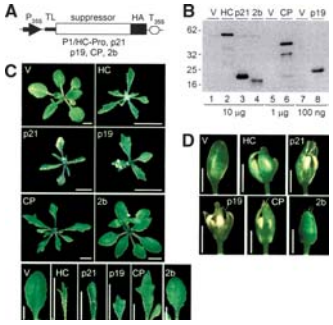
Disrupt RNAi pathway at different points, RISC formation, amplification, maintenance, systemic signal, etc.



Roth et al., 2004, Virus Research

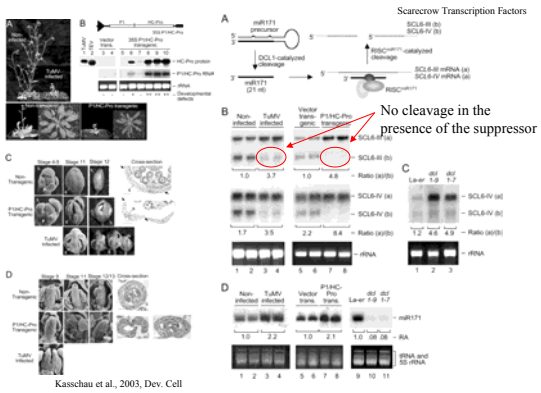
Disease responses associated with virus suppression of RNAi

Developmental defects induced by silencing suppressors from five viruses



Elisabeth J. Chapman et al. Genes Dev. 2004; 18: 1179-1186

Cold Spring Harbor Laboratory Press



Kasschau et al., 2003, Dev. Cell

Searching for miRNAs that target your gene of interest?

search the

Arabidopsis thaliana Small RNA Project — *An NSF 2010 project*

James C. Carrington and Kristin D. Kasschau

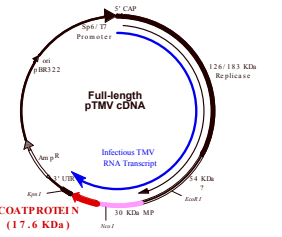
http://asrp.cgrb.oregonstate.edu/

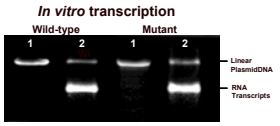
Reverse Genetics

Viral genome cloned into a bacterial plasmid and used to modify the genome.

In vitro transcription from plasmid template produces genomic viral RNA.

Rub inoculate viral RNA directly onto leaf surfaces to initiate infection.





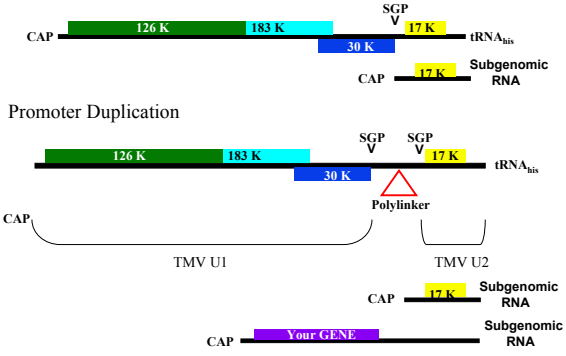
★ Virus Vectors:
(Rationale)
- High levels of protein expression (dsRNA)
- Their genomes can be modified
- Travel systemically throughout the plant
- Infection occurs rapidly

(Applications)

★ Functional genomics applications - the analysis and commercialization of gene and protein function.

★ Manufactured proteins and therapeutics - the manufacture of proteins and peptides for medicine, chemistry and agriculture.

Strategy for foreign gene expression from Tobacco Mosaic Virus Vector

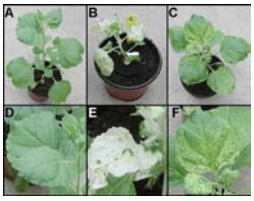


Promoter Duplication

★ Functional Genomic Studies Using Plant Virus Vectors

Silencing of host genes to study function.
Termed: Virus Induced Gene Silencing (VIGS).
Advantages: Rapid, No plant transformation, overcomes functional redundancy, works in different genetic backgrounds

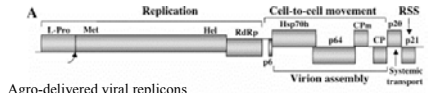
[Ruiz, et al, 1998].



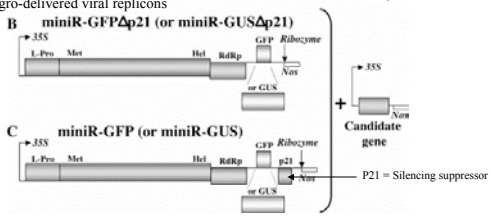
Phytoene desaturase Benedito et al., 2003

Diverse suppressors of RNA silencing enhance agroinfection by a viral replicon

Beet yellows virus genome

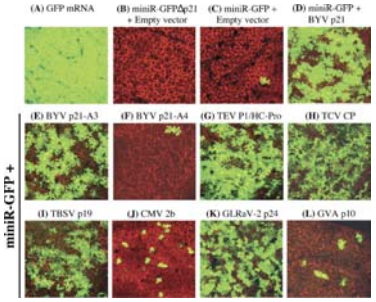


Agro-delivered viral replicons



Chiba et al., Virology, 2006

Diverse suppressors of RNA silencing enhance agroinfection by a viral replicon



Chiba et al., Virology, 2006