Websites to brush up on viral diseases

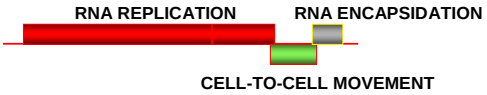
Barley yellow Dwarf

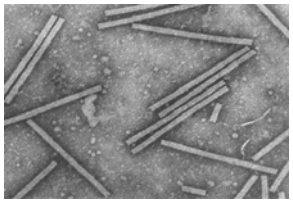
http://www.apsnet.org/education/LessonsPlantPath/BarleyYelDwarf/default.htm

Tobacco Mosaic Virus

http://www.apsnet.org/education/LessonsPlantPath/BlacklegPotato/default.htm

RNA genome of TMV: ~6,400 nucleotides, *three* genes, and *three* major functions





Virus Life Cycle

2 Genome uncoating, expression and replication

uncoating of virion

Host ribosomes translate (express) viral genome

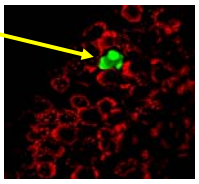
translation

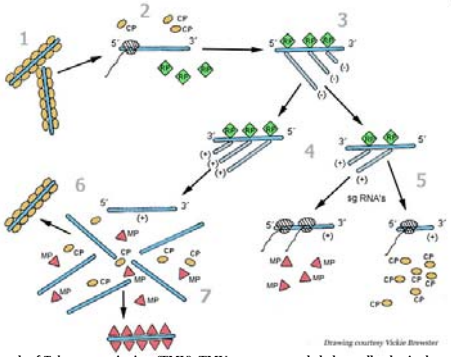
Gene Products:

replication

Replicase enzyme makes new copies of virus' genome

- Replicase (RNA polymerase) enzyme (gene product)
- New protein coat subunit (gene product)
- Cell to cell movement protein (gene product)





Drawing courtesy Vickie Brewster

The replication cycle of *Tobacco mosaic virus* (TMV). TMV enters a wounded plant cell to begin the replication cycle [1]. As the cost protein (CP) molecules are stripped away from the RNA [2], host ribosomes begin to translate the two replicase-associated proteins. The replicase proteins (RP) are used to generate a negative-sense (- sense) RNA template from the virus RNA [3]. This - sense RNA is, in turn, used to generate both full-length positive-sense (+ sense) TMV RNA [4] and the + sense subgenomic RNAs (sgRNAs) [5] that are used to express the movement protein (MP) and CP. The + sense TMV RNA is either encapsidated by the CP to form new TMV particles [6] or wrapped with MP [7] to allow it to move to an adjacent cell for another round of replication.

Interesting observations on viral plant diseases

Date first observed

- Sometimes the plant recovers!
  --- so called "Shock" diseases — 1950s
- Mild strain protects against disease by severe strain!
  --- "Cross-protection": used in Brazil for a citrus virus — 1970s
- Transgenic plants that express viral coat protein gene are resistant!
  --- Hypothesis of the day: Virus can't disassemble — 1980s
- Plants that express inversions of viral genes are resistant!
  --- Inverted gene has no product. Hmmmmm? — 1990s

Recovery from viral diseases

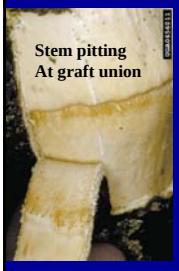
Blueberry Shock Disease

Blueberry bush without leaves on right is showing 'shock' for the first time. It will show the shock reaction for 1 to 3 years and may be symptom-free there after although it will still carry the virus.

Beet Necrotic Yellow Vein

Middle plant is recovering from initial symptoms caused by BNYVV

Citrus Tristeza Virus



From Japanese citrus production guide on the web:

"In areas where it is difficult to find a virus-free field, pre-inoculation with a mild CTV strain protects trees against infection with a severe strain of CTV."

Protection of papaya with the coat protein gene of papaya ringspot virus: a success story

Inoculation with *Papaya mosaic virus*

Transgenic **Non-transgenic**

Field trials with transgenic papaya in Hawaii

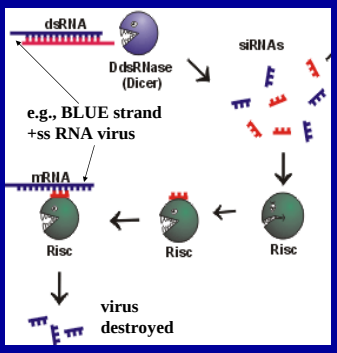
So, what is the biochemical mechanism that accounts for the 'interesting observations' on virus diseases?

RNA Silencing

RNA Silencing:

Plant and animal cells have a two step enzyme process to recycle RNA

1) DICER targets double stranded RNA molecules , and chops it into small pieces called silencing inducing RNAs (siRNAs, about 20 base pairs in length)



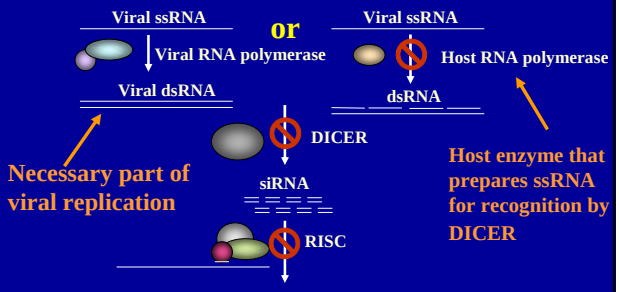
2) siRNAs become templates for the RISC enzyme complex. RISC uses the template to guide destruction of the original RNA molecule.

If RNA silencing is an efficient mechanism for destruction of double stranded RNAs,

then how do viruses succeed?

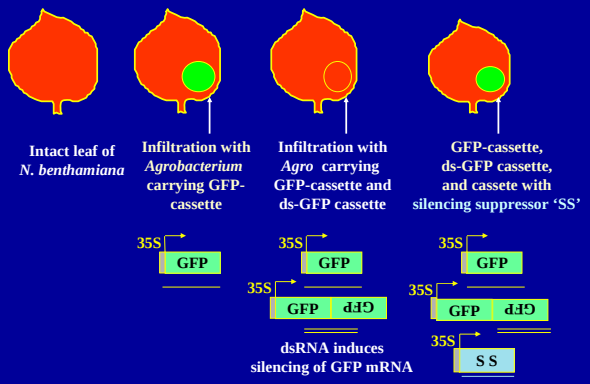
a. they outrun it
b. they suppress it
c. both

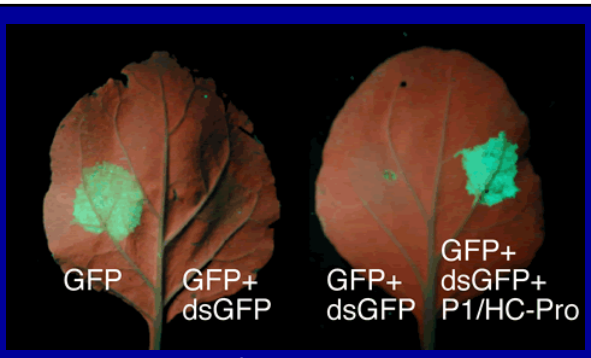
= Example of where a virus has been shown to suppress RNA silencing



Continued progression of viral infection

Identification of silencing suppressors





Introduction of inverted repeat of GFP results in silencing of GFP

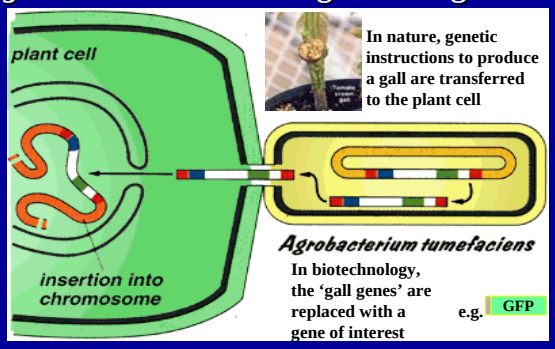
GFP

P1/HC-Pro is a viral suppressor of RNA silencing

S S

How easy is it to engineer plant resistance to a viral disease?

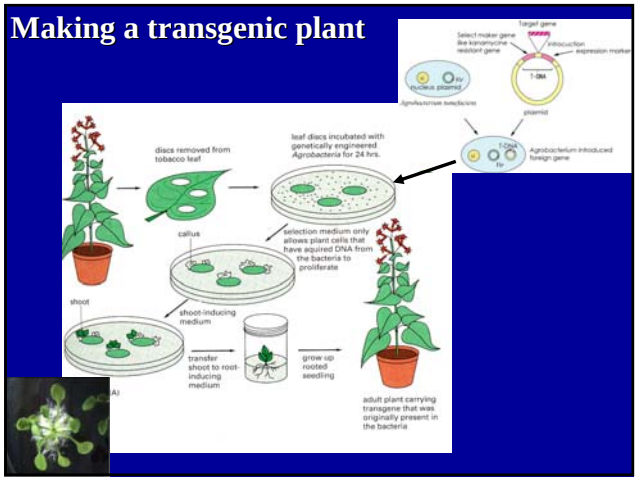
Agrobacterium: nature's genetic engineer



In nature, genetic instructions to produce a gall are transferred to the plant cell

In biotechnology, the 'gall genes' are replaced with a gene of interest e.g. GFP

Making a transgenic plant



Engineering viral resistance

1) Choose virus --- e.g. TMV

2) Choose sequence --- coat protein
http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=9626125

3) Make an inverted repeat of target sequence

genome bp 6001-6010 TMV cp

e.g.: ttgaaaatca-tgattttcaa ensures mRNA will double on itself, which prepares it for DICER

4) Attach promoter and insert into *Agrobacterium*

35S → TMV cp

5) Create and select transformants

Advantages of plant transformation over conventional breeding

- **Transformation permits transfer of resistance genes between sexually incompatible species**
- **It allows one to generate novel types of resistance including the cases when natural resistance does not exist**
- **Transformation is much less time consuming than breeding**
- **Single transformation procedure permits insertion of multiple genes**
- **Transfer of genes has no deleterious effects often associated with backcrossing**
- **Transformation works well for clonally propagated crops such as potato**

Course Content Since Midterm I

Theme 1: Disease dynamics

- How does disease develop in populations?
- Polycycle? Monocycle?
- What is 'r'? What is initial inoculum?
- What are the axis labels for a graph relating to speed of increase, how far it moves, or how many propagules are required to give a certain amount of disease?
- What does each type of curve look like?
- How does sanitation and host resistance influence disease progress?

Theme 2: Host resistance

- Two major types
- How do we know which type we have?
- How do we select for resistance?
- What is the gene-for-gene hypothesis?
- What are pathogen races?
- How do we manage resistance?

Theme 3:Basidiomycetes

- Describe the disease (life) cycle of a heteraceious, macrocyclic rust.
- How do we manage rust diseases with host plant resistance?

Theme 4: Bacteria

- What are they? How do we identify them?
- How do they cause disease?
- What is an epiphytic phase?
- What is the signicance of different types of secretion systems?