Life Cycle – DNA Plant Viruses



TOPICS:

1. Life Cycle of ssDNA plant viruses Geminiviridae



2. Life Cycle of RT Viruses RNA: transposons dsDNA: *Caulimoviridae*



3. Integration of viral genomes into plant genomes

ssDNA ViralZone DNA viruses RT viruses **RNA viruses** -viroids ds DNA പ്രവസ്തനംസ്വാനം ss DNA Transcribing DADADADADADA nn m NA SINGLE STRAND DNA VIRUSES Taxonomy Virions Genome stats Cell Receptors Order: Unassigned Rep-Encoding Single-Strand viruses-Inoviridae Genomoviridae Bacilladnaviridae Gemycircularvirus Fibrovirus Diatodnavirus New Gemyduguivirus Habenivirus Kieseladnavirus New Gemygorvirus Inovirus 99 Gemykibivirus Lineavirus Protobacilladnavirus Moved Gemykolovirus Plectrovirus Saetivirus Gemykrogvirus Circoviridae Gemykroznavirus Vespertilliovirus Circovirus Gemytondvirus Cyclovirus Gemyvongvirus Microviridae Bullavirinae Geminiviridae Parvoviridae Alpha3microvirus Becurtovirus Parvovirinae G4microvirus Begomovirus Amdoparvovirus Phix174microvirus Capulavirus Aveparvovirus Gokushovirinae Curtovirus Bocaparvovirus Bdellomicrovirus Grablovirus Copiparvovirus Chlamydiamicrovirus Eragrovirus Dependoparvovirus Spiromicrovirus Mastrevirus Erythroparvovirus Topocuvirus Smacoviridae New Protoparvovirus Turncurtovirus Tetraparvovirus Or O? Bovismacovirus New Densovirinae or ? Cosmacovirus New Nanoviridae Ambidensovirus or ? Dragsmacovirus New Babuvirus Brevidensovirus Nanovirus or ? Drosmacovirus New Hepandensovirus or ? Huchismacovirus Iteradensovirus or ? Porprismacovirus New

Penstyldensovirus

4/24/2019



SINGLE STRAND DNA VIRUSES



4/24/2019

Pleolipoviridae

- Alphapleolipovirus
 Betapleolipovirus
 Gammapleolipovirus

 Spiraviridae
 - Alphaspiravirus

Family: Geminiviridae

- Circular, single stranded DNA genome
- Contain the smallest known plant viruses 2800 nt (to 5200 nt)



 Morphology – unique particle shape consists of a fusion of two incomplete icosahedra (18x30 nm), geminate particle



Geminiviridae:

- 1. All known vectors in the Order *Hemiptera*
- Genus Mastrevirus
- Genus Becurtovirus, Curtovirus
- Genus Topocuvirus
- Genus Grablovirus
- Genus Begomovirus
- Genus Eragrovirus, Turncurtovirus
- Genus Capulavirus

leafhoppers

leafhoppers

treehopper treehopper

opper 🔤

whiteflies ??

aphids





All are monopartite except *Begomovirus* which has some members with bipartite genomes



Family Geminiviridae

Genome:

Monopartite or Bipartite

Monopartite: single stranded, circular DNA 2,500-3,000 nt;

Bipartite: two single stranded, circular DNA molecules, ~2500 nt each segment for a total ~5000 nts





Symptoms associated with geminivirus infection



Maize streak virus (Mastrevirus)



Cotton leaf curl virus (Begomovirus)



Abutilon mosaic virus (Begomovirus)













Begomoviruses In Florida

Wild plants		Viruses in Sida spp. Sida golden mosaic virus (1993) Sida golden mosaic Florida virus (1995 Sida golden mottle virus (2004) Sida golden yellow vein virus (2012) Sida golden mosaic Yucatan virus (2013)	
	* * * *	Macroptilium yellow mosaic Florida virus (2003 Dicliptera yellow mottle virus (1996) Chenopodium leaf curl virus (2010) Euphorbia mosaic virus (2011))
Ornamentals		Abutilon mosaic virus - 1995 Jatropha mosaic virus (2011)	
Vegetable crops	****	Tomato mottle virus - 1989 Bean golden yellow mosaic virus - 1993 Cabbage leaf curl virus - 1995 Tomato yellow leaf curl virus - 1997 Cucurbit leaf crumple virus - 2007	Red –Introduced Blue – Prob. native

Geminivirus Genome Organization

7 Genera: Becurtovirus Begomovirus Curtovirus Eragrovirus Mastrevirus Topocuvirus Turncurtovirus

- Similar arrangement of genes
- Most have monopartite genomes



Geminivirus Genome Organization

7 Genera: Becurtovirus Begomovirus Curtovirus Eragrovirus Mastrevirus Topocuvirus Turncurtovirus



- Geminivirus ORFs are on both virion sense and complementary sense strands;
 ORFs overlap and share the same nucleotides
- Arrangement of genes is a little different in the bipartite genomes

Geminivirus Genes and Functions

Like other viruses, each protein may have many functions



How do geminiviruses replicate?

- Begomoviruses have a circular covalently closed ssDNA genome
- But they produce a dsDNA as an intermediate step in the replication of the genome
- Replication is in the plant cell nucleus
- A lot of dsDNA is present in infected plants, but relatively little ssDNA (and relatively little protein)
- Replication occurs primarily in phloem parenchyma cells, but replication can occur in other cells



Geminiviruses Commandeer Host Cells

Healthy Cell Cycle:



Common strategy for mammalian tumor viruses, plant DNA viruses: Control the cell cycle so that the infected cell cannot progress to G₂ and stays in S phase (DNA synthesis phase)

G₁: the cell grows larger, copies organelles, and makes the molecular building blocks it will need in later

In mammalian cells, a key cell cycle regulator is the <u>retinoblastoma tumor</u> <u>suppressor protein</u> (RB).

Healthy Cells:

RB protein holds cells in G_1 phase (cell is transcriptionally active). RB represses onset and progression into S phase by interacting with a wide range of cell cycle-related proteins.

Infected Cells:

Viral encoded Rep protein binds RB, so that the cell progresses from G_1 into S phase but does not progress into G_2 .

... studies suggest that mammalian tumor viruses and DNA plant viruses (geminiviruses, nanoviruses) employ similar strategies to control the host cell cycle.

- Geminiviruses reprogram host transcriptional controls to induce quiescent plant cells to reenter the cell cycle and regain the capacity to support high levels of DNA replication
 - Geminivirus Rep (one of its many functions) ===> binds host RB PLUS.....
 - Proliferating-cell nuclear antigen (PCNA)

 keeps the cell in S phase
 transcription of PCNA is activated by Rep so PCNA accumulates in infected
 cells and this helps keep the cell in S phase

PCNA protein





Red/brown color indicated presence of PCNA protein

The geminivirus life cycle:

Viral **single-stranded DNA (**ssDNA) is released from virions and copied to generate **double-stranded DNA** (dsDNA) using host enzymes and cofactors. The dsDNA, which assembles with nucleosomes, is transcribed by host RNA polymerase II, resulting in the production of replication initiator protein (Rep).

Rep initiates rolling-circle replication (RCR) by introducing a nick into a viral dsDNA molecule to generate a free 3'-hydroxyl end that primes ssDNA synthesis, leading to displacement of the parental strand (inset).

The **ssDNA is converted to dsDNA** and re-enters the replication cycle. Replication then transitions to recombination-dependent replication (RDR), initiated by recombination between a partially replicated ssDNA and a closed, circular dsDNA to form a looped molecule that serves as a template for both ssDNA and dsDNA synthesis (inset).





Geminivirus virions and inclusions

Geminivirus virions accumulate in the nucleus, many infections are confined to phloem tissues.





The geminivirus life cycle.

Later in infection, **Rep represses its own transcription**, leading to activation of transcriptional activator protein (TrAP) expression, which in turn activates coat protein (CP) and nuclear shuttle protein (NSP) expression.

Encapsidation: Circular ssDNA can then be encapsidated by CP into virions in the nucleus, which are available for whitefly acquisition.

Cell to cell movement: NSP binds to viral DNA and moves it across the nuclear envelope, where movement protein (MP) traffics it across a plasmodesmata.

It is not known whether viral DNA moves as ssDNA versus dsDNA or as a linear versus a circular molecule.





(a) RCR. The viral circular ssDNA is released from the virion (yellow) into the nucleus. The host DNA polymerase synthesizes the complementary strand, yielding circular covalently-closed dsDNA. This dsDNA serves as a template for bidirectional transcription of the early leftward (Rep) and the late rightward (coat protein) genes. Viral mRNAs are transported to the cytoplasm. Following translation, Rep moves to the nucleus to initiate replication of the viral dsDNA by a rolling circle replication (RCR) mechanism. Rep (in yellow) nicks the virion strand in the origin of replication and recruits the host DNA polymerase to extend 3'-end of the cleaved virion strand on the complementary strand template. As the extension progresses, the polymerase complex, associated with Rep covalently linked to the 5'-end of the virion strand, displaces the virion strand. After one or more rounds of replication on the circular complementary strand template, Rep nicks and religates the displaced virion strand extended by one or more copies of the newly-synthesized virion strand and thereby releases one or more copies of circular ssDNA. The resulting circles can re-enter the replication cycle or get packaged into virions;

b) **RDR.** The circular covalently-closed dsDNA is invaded by a short viral DNA primer. The primer is extended by the host DNA polymerase on the circular viral template strand. After (or during) one or more rounds of replication, the newly-synthesized linear ssDNA gets fully or partially converted to linear dsDNA by the same (or another) DNA polymerase complex. Thus, RDR generates a heterogeneous population of linear dsDNAs. The long linear dsDNAs that harbor two or more origins of replication are transcribed by Pol II in both orientations to generate viral mRNAs. Following translation, Rep initiates replication of the long linear dsDNA with two or more origins of replicational release of ssDNA from the multimeric linear dsDNA generates circular ssDNA that can re-enter the replication cycle or get packaged.



Short video on Geminivirus replication: www.youtube.com/watch?v=EPoNXpPb80U

Lecture on DNA virus replication: www.youtube.com/watch?v=ezPtQwXcmQs

Multiple functions of the Geminivirus *Rep*:

Rep (Replicase associated protein) has many functions:

- Binds RB
- Bind to viral DNA and nicks the rolling circle essential for DNA replication



Transcription of mRNA for gene expression

- Transcription is bidirectional with independently-controlled transcripts.
- Transcripts are produced off the dsDNA replication intermediate



Genome and transcriptome of MYMV. Rightward- and leftward-oriented ORFs, encoded on the viral and complementary stands, respectively, are shown as thick arrows with the names of their products. The major transcription units mapped in this work are depicted as thin arrows. The minor DNA-B transcript is shown by a dotted line and the intron found in the major BC1 transcript by a dashed line.

From: Shivaprasad et al <u>J Virol. 2005 July; 79(13): 8149–8163.</u>

2. Life cycle of Retro transcribing dsDNA viruses



Genomes:

dsDNA or ssRNA

REVERSE-TRANSCRIBING VIRUSES



Caulimoviridae

- Caulimoviruses are the plant homologues of mammalian retroviruses, like HIV-1.
- Caulimoviruses normally exist in the plant nucleus, they are capable of integrating into the host chromatin.



HIV – Human immunodeficiency virus



Banana streak virus (BSV) (Badnavirus) in plantain



Blueberry red ringspot virus (Caulimovirus) in blueberry





Cacoa swollen shoot virus (CSSV) (Badnavirus) in cacoa Citrus mosaic virus (Badnavirus)

Biological Properties

- Many virus species are spread by vegetative propagation.
- Geographic distribution: many species, widely distributed.

Tungrovirus and Badnavirus: predominately in the subtropics, and tropics
 Caulimovirus, Cavemovirus, Petuvirus, Rosadnavirus, and
 Soymovirus: predominately in the temperate regions

Capsid:

Isometric 45-50 nm dia (T=7) or Bacilliform (24-30 nm in dia. 60 – 900 nm)

- Virions contain a single molecule of <u>open circular dsDNA</u>
 7.2 to 8.1 kbp.
- Each strand of the DNA genome has discontinuities (breaks) at specific places, the number and location varies among the genera.

Genome:

- Monopartite
- 1 to 8 ORFs, depending on the genus.
- The functions of virus-encoded proteins common to all genera are the capsid protein (CP), an aspartate protease, a reverse transcriptase and a ribonuclease H.





Genome Organization

- Discontinuities: DNA strands are not continuous (have one or more breaks in the ds genome)
- Discontinuities allow for binding of reverse transcriptase
- One DNA strand serves as the template
- Internal initiation for viral mRNAs
- VERY complex replication strategy and much is still not clear

Yellow circles – dsDNA genome; green – discontinuities; red – promoters; Inner blue arcs - orfs, Outer blue arcs - mRNAs



Genus Caulimovirus – Genome Organization



Figure 2 Circular (top) and linearized (bottom) genome maps of *Cauliflower mosaic virus* (CaMV). ORFs or ORF segments encoding a protein are represented by boxes shaded in different patterns according to the different putative functions of the genes (vertical lines for the putative movement protein, slanted lines for the capsid protein and horizontal lines for the reverse transcriptase protein). The symbols used are the following:
■ movement protein active site, * RNA binding site, ◇ protease active site, ◆ reverse transcriptase active site, △ RNAse H consensus sequence. The map starts at the intergenic region of the circular genome for convenience, the arrow shows the position of the promoter and the number 1 indicates the origin of DNA replication.

- CaMV 35S promoter is a strong promotor (responsible for the production of one very large RNA (35 s, full genome length plus a bit more)),
- It is often used to express foreign genes in plants (genetic engineering)
- Transformation construct:





Flower transformed with *gfp* gene, photographed under UV light

Virus genus	СР	RT	RH	AP	CTC	HC
Caulimovirus	IV	v	v	v	Ι	П
'SoyCMV-like'	IV	v	v	v	Ia	ND
'CVMV-like'	I	III	III	III	I	ND
'PVCV-like'	п	II	II	п	I	ND
Badnavirus	III	III	III	III	III	ND
'RTBV-like'	III	III	III	III	ND	ND

Table 4 Properties of gene products of Caulimoviridae: gene functions^a

CP, coat protein; RT, reverse transcriptase; RH, ribonuclease H; AP, aspartate proteinase; CTC, cell-to-cell spread (movement) protein; HC, insect vector helper protein.

"Open reading frame encoding gene function (Figure 1).

Viral Coded Proteins:

- CP coat protein
- RT Reverse transcriptase
- RH RNase H
- AP Aspartate Proteinase
- CTC cell to cell movement
- HC helper component (vector transmission)



Genome Replication Cycle:

- 1. DNA is released from the virion into the nucleus,
- 2. Discontinuities (breaks) are sealed, dsDNA coils into mini-chromosomes.
- Host polymerase (RNA Pol II) uses the 35S promoter to transcribe a genome-length mRNA (35sRNA) then the 19s RNA is transcribed.
- The 35sRNA is transported from nucleus into the cytoplasm where some of the 35sRNA is translated by host ribosomes to produce the RNA dep DNA polymerase (aka Reverse Transcriptase).


Genome Replication Cycle:

- 5. Reverse Transcriptase reads the same strand of 35sRNA and transcribes a ssDNA (forming a RNA-DNA intermediate).
- Viral encoded RNaseH removes the RNA strand, and the now ssDNA is transcribed by Reverse Transcriptase to produce a second strand of DNA (you now have dsDNA) in the viroplasm.
- 7. Or ssDNA may return to the nucleus



35sRNA serves as an mRNA and as template for DNA synthesis

mRNAs and Protein Production:

35s RNA contains most of the ORFs (35s RNA is a polycistronic mRNA)

19sRNA codes for a protein that controls translation re-initiation of major open reading frames



CaMV inclusion body in epidermal cell of *B. campestris* (mustard), showing virus particles embedded in the densely-staining granular matrix. This inclusion body is the viroplasm.

Bar represents 500 nm. (Photo courtesy A. Allison).



https://www.youtube.com/watch?v=7oyp1zIIWmM

video animation of HIV replication: Transcription, DNA replication Encapsidation Etc...

REVERSE-TRANSCRIBING VIRUSES



REVERSE-TRANSCRIBING VIRUSES

Caulimoviridae Badnavirus Caulimovirus Cavemovirus Petuvirus Rosadnavirus	Retroviridae Orthoretrovirinae Alpharetrovirus Gammaretrovirus Deltaretrovirus
 Solendovirus Soymovirus Tungrovirus 	PTLV-1 PTLV-2 PTLV-3 PTLV-4
Retrotransposons Belpaoviridae New Semotivirus Move Metaviridae Metavirus Errantivirus Pseudoviridae Memivirus Sirevirus Sirevirus	Retrotransposons: Organization in families and genera based on sequence similarity and order of encoded gene products
EGEND: Human Vertebrate	Eukaryotic Fungi Invertebrate Plant Archeabacteria Bacteria

REVERSE-TRANSCRIBING VIRUSES



2. Retro Transcribing Viruses -ssRNA

Long terminal repeat retrotransposons - LTR retrotransposons (LTR-RTNs)

- Do not form infectious particles that leave the cells and therefore only replicate inside their genome of origin
- Range in size from 100 to 25,000 bp
- Found in high copy numbers in genomes of a wide range of organisms (animals, fungi, protista, plants)

- Evolutionarily related to retroviruses. Its believed that retroviruses evolved from *Gypsy* LTR-RTNs after the acquisition of an envelope gene.

Pararetroviruses and Retrotransposons

- Much of the replication cycles of pararetroviruses and retrotransposons are similar.
- Both have long terminal repeat (LTR) sequences and coding regions with sequence motifs for reverse transcriptase (RT), ribonuclease H (RH), and aspartate protease (AP) – similar to retroviruses that are capable of integration.
 - Replication involves an integrase (int) gene, which mediates insertion of the element into the host chromosome.



3. Integration of viral genomes into plant genomes

 Integrants of three Caulimoviruses: Banana streak virus (BSV), Tobacco vein clearing virus (TVCV), and Petunia vein clearing virus (PVCV), can generate infections in certain hybrid plant hosts in response to stress.

> Ex. *Musa spp*. during tissue culture, *Banana streak virus* infections can arise in healthy appearing plants from integrated BSV sequences)

 As the number of sequenced plant genomes increases, so does the number of examples of integrated viral sequences.



Banana streak virus (BSV) (Badnavirus)

Pararetrovirus sequences in plant genomes

- Species of *Caulimoviridae* integrate into the genome and are passed vertically to the next generation in the genome (seed transmission?)
- Plant geneticists find integrated viral genomes as they sequence plant genomes and refer to them as <u>Endogenous pararetroviruses</u> (EPRVs)
- Sequences also found in viral metagenomic studies that use siRNAs
- Not all integrated *Caulimoviridae* sequences have been shown to initiate virus infections (silent vs active)



Christina Staginnus¹ and Katja R. Richert-Pöggeler^{2,3}

Host plant	Structural classification	Activatable EPRVs	Non-activatable EPRVs	Corresponding episomal virus
Musa balbisiana	Badnavirus	BSOLV EPRV, BSGFV EPRV-EPRV 7	BSMysV, BSImV, BSOLV, BSGFV-EPRV 9	BSMysV, BSImV, BSOLV, BSGFV
<i>Oryza sativa (japonica, indica)</i> also: <i>Oryza</i> sp.	Tungrovirus		ERTBV-A; ERTBV-B; ERTBV-C	RTBV
Petunia hybrida also: Petunia sp.	Petuvirus	ePVCV	ePVCV	PVCV
Nicotiana edwardsonii, Nicotiana tabacum also: Nicotiana sp.	Cavemovirus	TVCV	TVCV	TVCV ^a
Nicotiana sylvestris, N. tabacum also: Nicotiana sp.	Cavemovirus	-	NsEPRV (TEPRV, TPVL)	TVCV ^a
Nicotiana tomentosiformis, N. tabacum also: Nicotiana sp.	Cavemovirus	-	<i>Nto</i> EPRV	TVCV ^a
Solanum tuberosum	Cavemovirus	8 	Sotul, Sotulli	TVCV ^a
Solanum subsection Lycopersicon	Cavemovirus	-	LycEPRV	TVCV ^a

Table 1 Selection of EPRVs identified in plant genomes, their current nomenclature and their homologous exogenous viruses

EPRV: <u>Endogenous pararetrov</u>irus sequence Activatable: can release a replication-competent viral genome Episomal virus: a replicating virus

Staginnus et al 2007

Seed Transmission and Pararetroviruses

- if sequence is integrated but not active referred to as an EPRV - If virus infection is shown then refer to that by the virus name

Table 1 Selection of EPRVs identified in plant genomes, their current nomenclature and their homologous exogenous viruses

Host plant	Structural classification	Activatable EPRVs	Non-activatable EPRVs	Corresponding episomal virus	References
Musa balbisiana	Badnavirus	BSOLV EPRV, BSGFV EPRV-EPRV 7	BSMysV, BSImV, BSOLV, BSGFV-EPRV 9	BSMysV, BSImV, BSOLV, BSGFV	[3, 6, 7, 16–19]
Oryza sativa (japonica, indica) also: Oryza sp.	Tungrovirus	-	ERTBV-A; ERTBV-B; ERTBV-C	RTBV	[8]
Petunia hybrida also: Petunia sp.	Petuvirus	ePVCV	ePVCV	PVCV	[9, 20]
Nicotiana edwardsonii, Nicotiana tabacum also: Nicotiana sp.	Cavemovirus	TVCV	TVCV	TVCV ^a	[21]
Nicotiana sylvestris, N. tabacum also: Nicotiana sp.	Cavemovirus	-	NsEPRV (TEPRV, TPVL)	TVCV ^a	[22]
Nicotiana tomentosiformis, N. tabacum also: Nicotiana sp.	Cavemovirus	-	NtoEPRV	TVCV ^a	[14]
Solanum tuberosum	Cavemovirus	-	Sotul, SotuIII	TVCV ^a	[10]
Solanum subsection Lycopersicon	Cavemovirus	-	LycEPRV	TVCV ^a	[11]

BSOLV Banana streak virus Obino I'Ewai species, BSMysV Banana streak virus Mysore species, BSImV Banana streak virus Imove species, TVCV Tobacco vein clearing virus

^a However, existence of an independent external virus is uncertain

Arch Virol (2009) 154:1189-1193 DOI 10.1007/s00705-009-0412-y

Example Banana streak virus:

 Every Musa spp. examined to date contains BSV DNA integrated into its chromosomes, either as tandem repeats of full length BSV genomes or as multiple copies of partial BSV genomes.

Mechanism of Integration:

- Integration of viral sequences into the host genome involves a recombination event. If there is no viral sequence present in the host genome, the recombination is **non-homologous**.
- If there are viral sequences already present, the recombination would be homologous.
- It is possible that homologous recombination leads to the formation of the complex integrants (as in the case of BSV).
- Most sequences have probably integrated into the host genome by nonhomologous recombination.

Integration of DNA viruses into Plant Genomes

- The number of integrant loci is variable:
 Ex. Its low for BSV and PVCV, but is high for TVCV.
- The structure of integrants can be complex (esp in the case of nonhomologous recombination)
- Release of integrant into episomal virus: thought to be by recombination and/or reverse transcription
- It appears that integration of viral sequences is widespread in the plant kingdom and has been occurring for a long period of time.

Other plant viral genome sequences can be found in plant genomes:

Plant virus sequences in plant genomes:

Caulimoviridae



How can RNA viruses integrate into host DNA chromosomes?

```
Involvement of a retrotransposon?
Viral sequence came from the host originally?
```

Baltimore System for Organizing Virus Families and Genera

