Plant Virus replication

(Plant virus infection process)

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INTRODUCTION

- **Virus infection & replication – A synonym**
- **Virus is a nucleo-protein having RNA or DNA as a genetic material.**
- **RNA or DNA may be ss or ds, ssRNA may be +ve or –ve sense.**
- **Most of plant virus infect a limited number of different plant species and a few have a wide host range.**
- **Viruses do not produce any kind of reproductive structure, they multiply by using host machinery.**

CONCEPT

- Two separate system involved in synthesis of protein and nucleic acid
- These two components then combine & form a nucleo-protein particle
  - Thus involves two inter-related biosynthetic mechanisms of the host
- The n-acid directs the synthesis of progeny n-acid and this in turn,
  - In association with host ribosome's form proteins by multistep process.

Host functions used by plant viruses

- Components for virus replication
  - (amino-acids & nucleotides synthesized by host cell metabolism for virus)
- Energy:
  - involved in polymerization of viral proteins and n-RNA synthesis as nucleoside triphosphate
- Protein synthesis
  - Viruses use ribosomes, tRNA and associated enzymes & factors of host
  - Involve 80S cytoplasmic ribosome system

- Nucleic acid synthesis
  - Generally almost all viruses code for an enzyme or enzymes involved in synthesis of their n-acids but may not contribute the polypeptides involved
  - Some viruses need host polymerases like Caulimoviruses the viral DNA enters host cell nucleus and transcribe into RNA form by host's DdRp II.
  - In majority of RNA viruses, RdRp is involved in replication complex.
- Structural components of the cell
  - Membrane bound complexes (endoplasmic reticulum; cytoskeleton

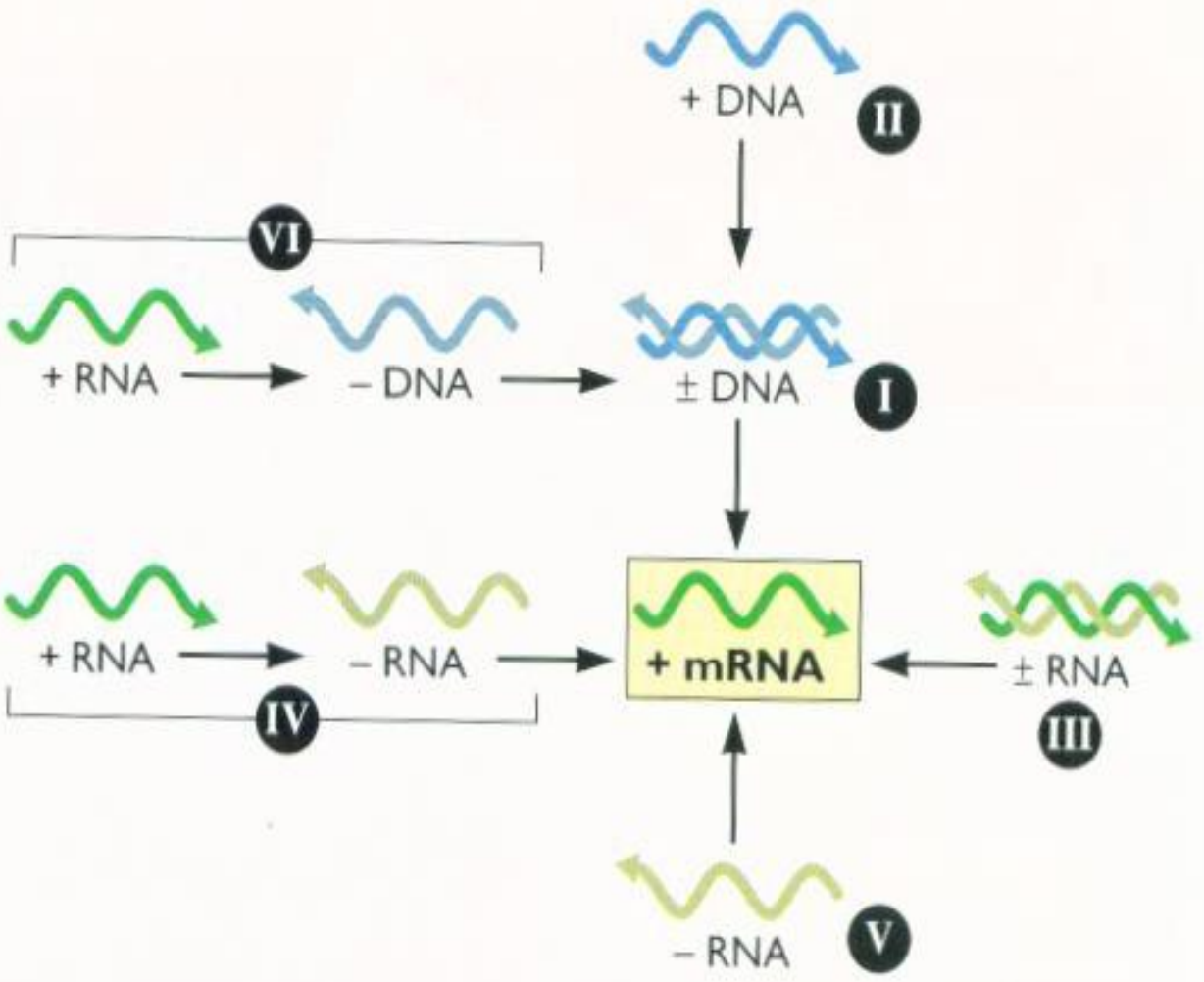
REPLICATION STRATEGY

- Virus absorption into host cells through wounds
- Virus entry into host cells and to replication sites in the cells
- Uncoating or disassembly of n-acid
- Translation and transcription of the viral genome:
  - Replication of viral n-acid
    - Viral genome either translated directly if +ve ssRNA or mRNAs
  - Gene products like replicases or viral coat protein and other proteins
- Assembly or maturation of new virus particles
- Transport o infective particles from cell to cell and over long distances with in the plants

VIRAL GENOME EXPRESSION

- I: is transcription of dsDNA usually by host-DdRP
- II: is transcription of ssDNA to give ds template for I (Gemini viruses)
- III: is transcription of dsRNA usually by virus coded RdRp (Reoviruses)
- IV: is replication of +ve stand RNA via –ve stand template by virus coded RdRp; the viral (+) strand is often the template for the early translation (=ve sense RNA viruses)
- V: is the transcription o _ve sense virus genome by virus coded RdRp (Tospoviruses)
- VI: is the reverse transcription of RNA stage of retro and para-retroviruses leading to dsDNA template for mRNA transcription.

Figure 1.10 The Baltimore classification. All viruses must produce mRNA that can be translated by cellular ribosomes. In this classification system, the unique pathways from various viral genomes to mRNA define specific virus classes on the basis of the nature and polarity of their genomes.



MAJOR EVENTS

- Entry
- Replication
- Movement

MODE OF ENTRY

- ✓ Wounds
  - Mechanically TMV, BCMV, PVX, PYDV,
  - Vectors PLRV,TLCV, WTV
- ✓ Pollen Grain Prunus necrotic ring spot, Tobacco ring spot of soybean Barley stripe mosaic virus
- ✓ Seeds or vegetative propagation BCMV, CMV, Pea stripe virus
- ✓ Pinocyte CPMV, Pea enation mosaic Cowpea Chloretic mottle virus
- ✓ Fungal parasite TNV , Barley yellow mosaic virus Tobacco stunt virus
- ✓ Ectodesmata TMV
- ✓ Epidermal hair TMV

UNCOATING OF VIRUS PARTICLE

- Uncoating is removal of virus protein which is their around the nucleic acid.
- The ability of uncoating depends upon structure of virus coat protein.

Different experiments indicating uncoating of viral RNA

- TMV multiplication detected some hours earlier when inoculation is done by RNA.
- Symptoms appears several hours earlier if naked TMV RNA is used as inoculum.
- Infectible sites produced by TMV RNA inoculum become resistant to UV radiation immediately after inoculation.

MECHANISM OF REPLICATION

- **Production of new RNA molecule identical to template RNA is called Replication.**
- **Viral RNA performs two function**
  - **It serves as mRNA, immediately binds to ribosome and directs the synthesis of virus specific proteins.**
    - **Among which one protein inhibits host protein and host RNA synthesis, and**
    - **other protein is specific viral replicating enzyme i.e. RNA polymerase or replicase.**
  - **Parental viral RNA is then displaced from ribosome, triggers the replication of viral RNA during which it behaves as a template or plus strand for synthesis of minus strand in presence of specific replicase already synthesized.**

RNA VIRUS STRATEGIES

RNA -> RNA

RNA-dependent RNA polymerase

RNA -> DNA

RNA-dependent DNA polymerase
- reverse transcriptase

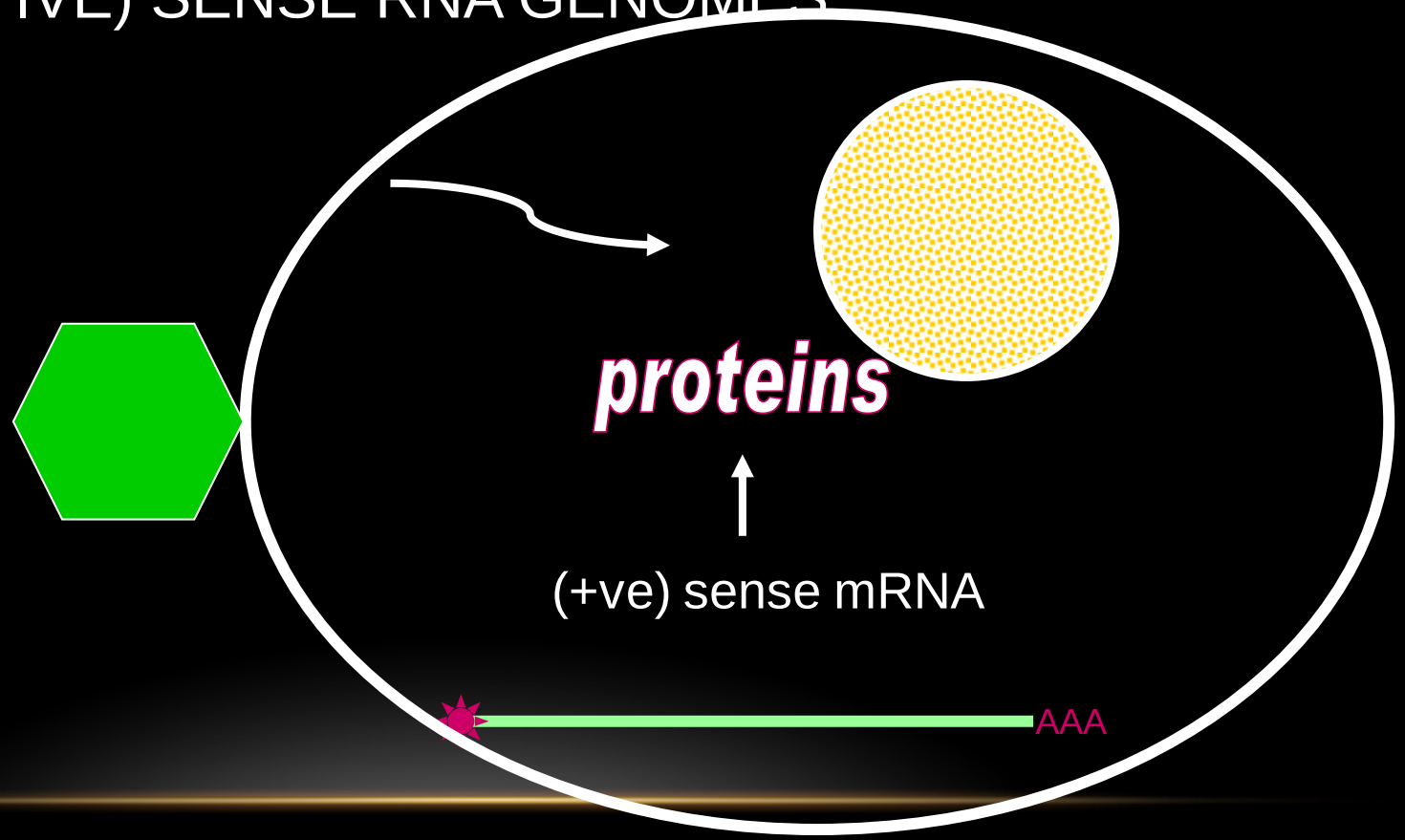
Host cell DNA -> RNA

DNA-dependent RNA polymerase

ALL RNA VIRUSES CODE FOR A POLYMERASE

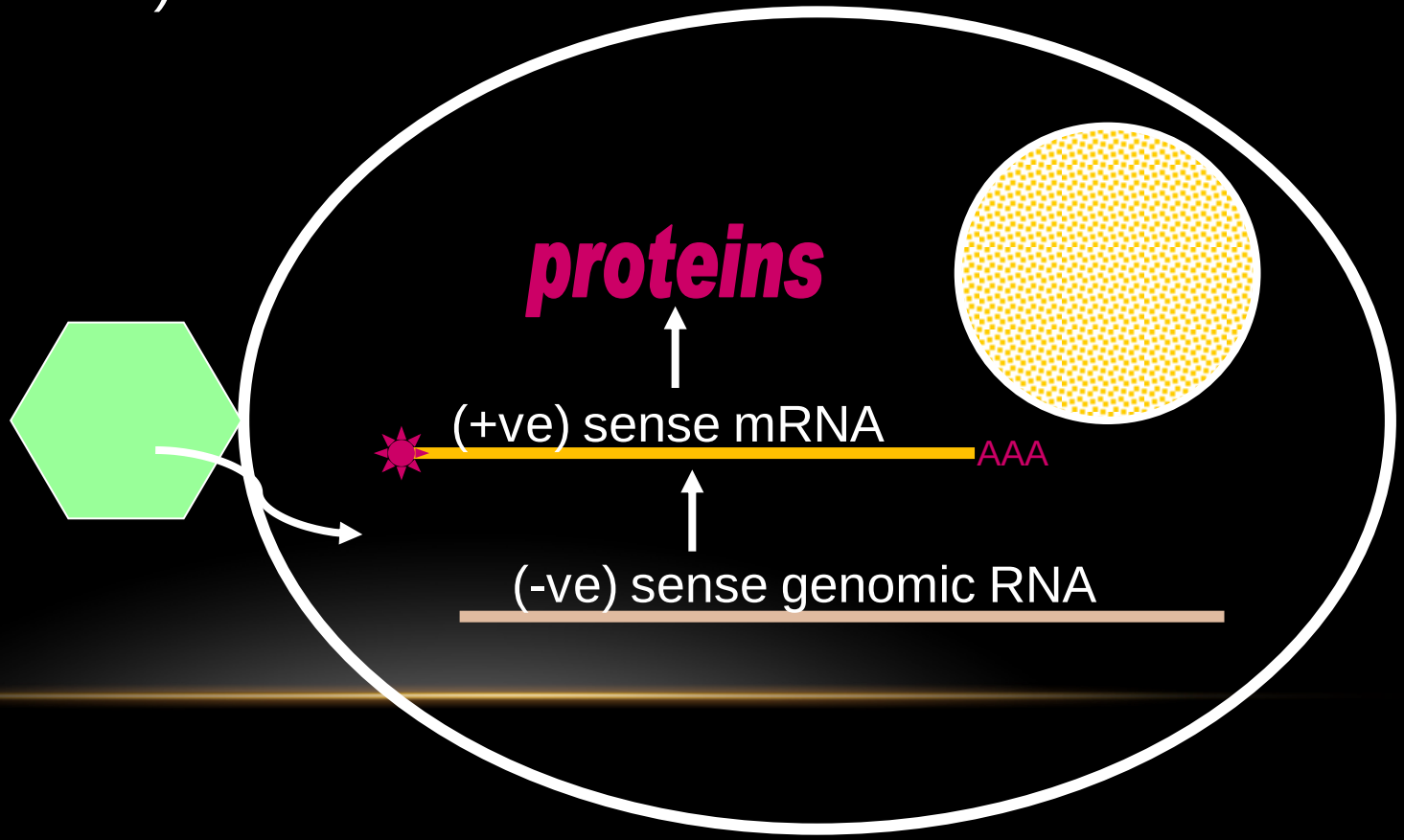
NEED TO MAKE MRNA

PLUS (POSITIVE) SENSE RNA GENOMES



NEED TO MAKE MRNA

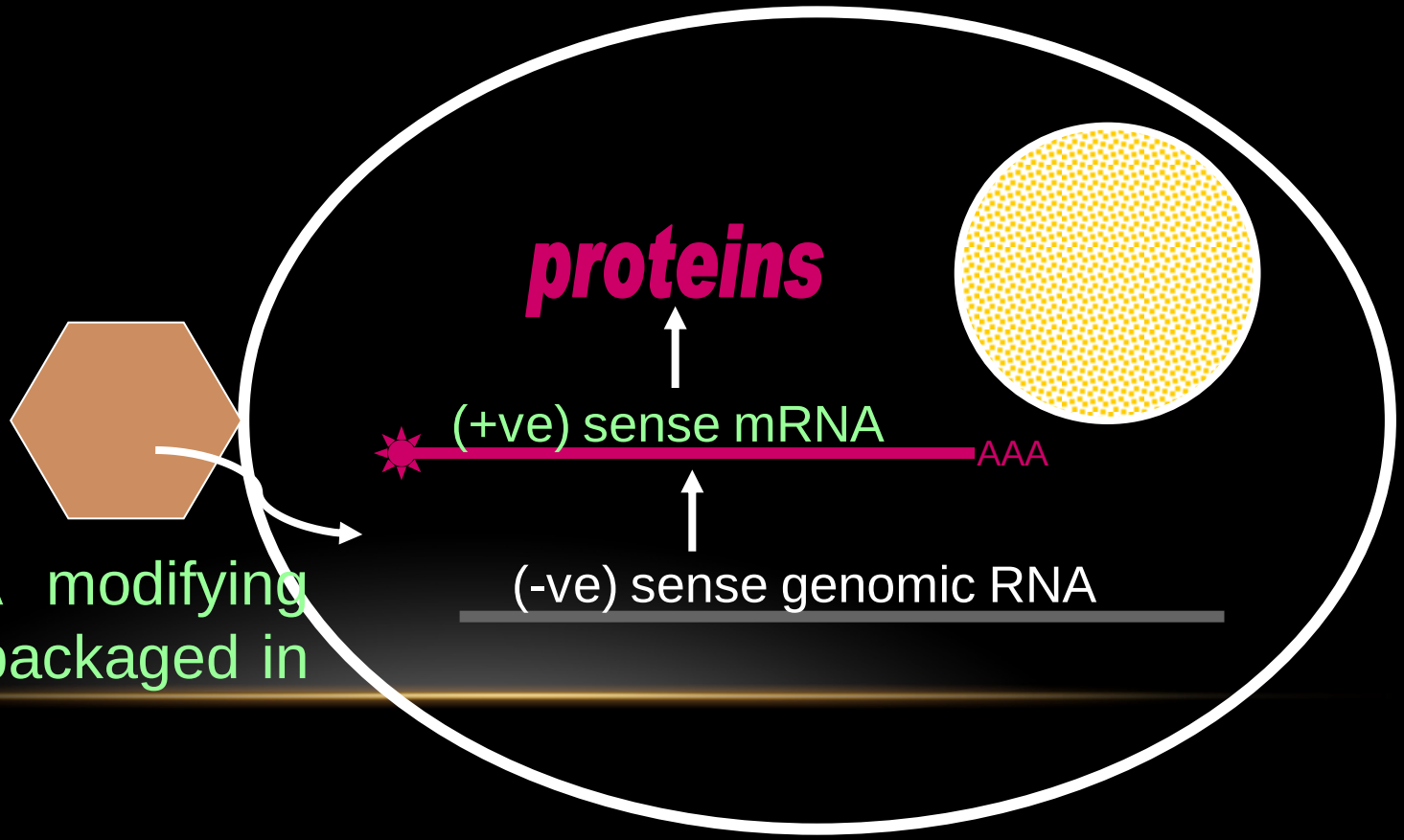
MINUS (NEGATIVE) SENSE RNA GENOMES



NEED TO MAKE mRNA

MINUS (NEGATIVE) SENSE RNA GENOMES

RNA polymerase must be packaged in virion

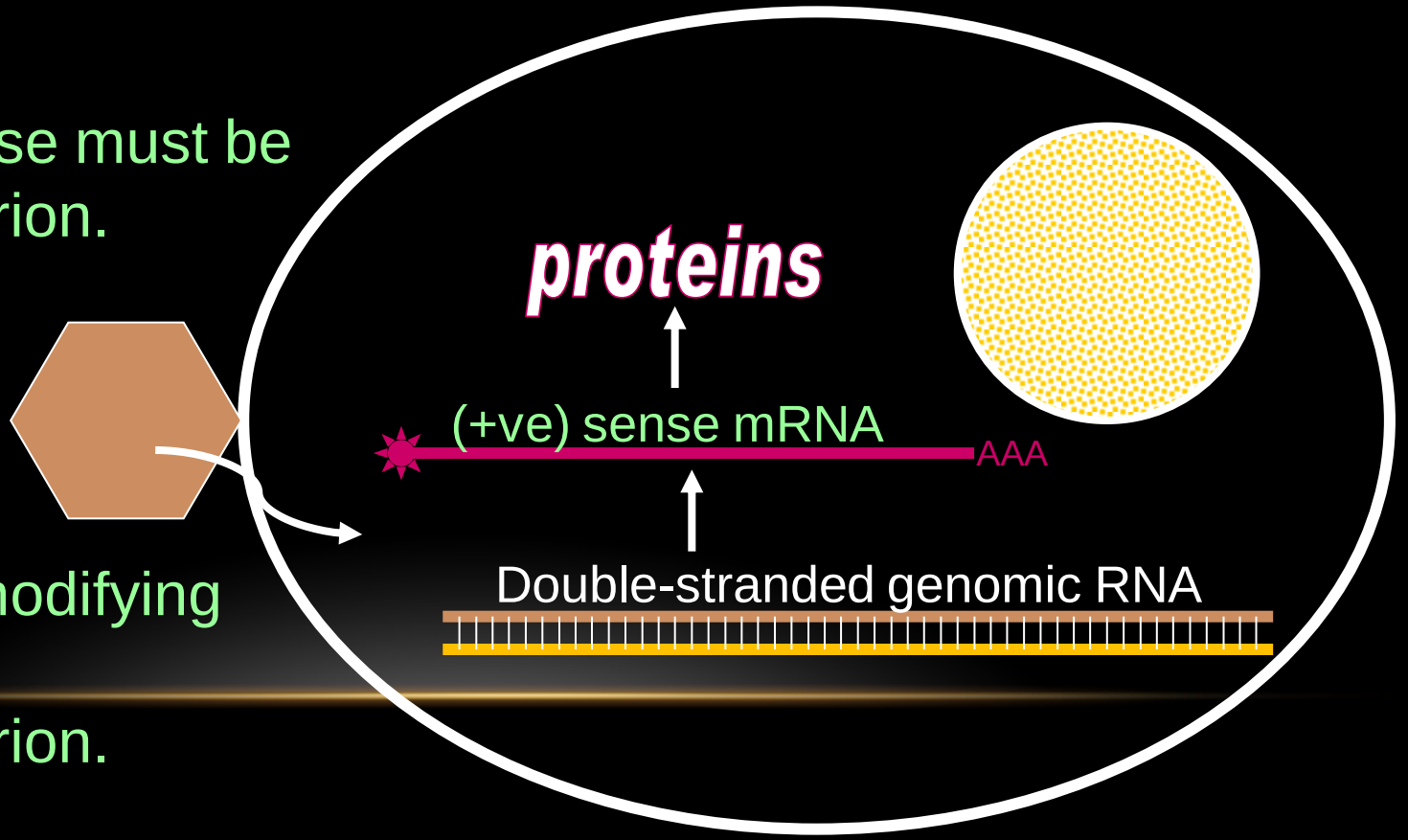


If used, RNA modifying enzymes are packaged in virion.

NEED TO MAKE mRNA

DOUBLE-STRANDED RNA GENOMES

RNA polymerase must be packaged in virion.

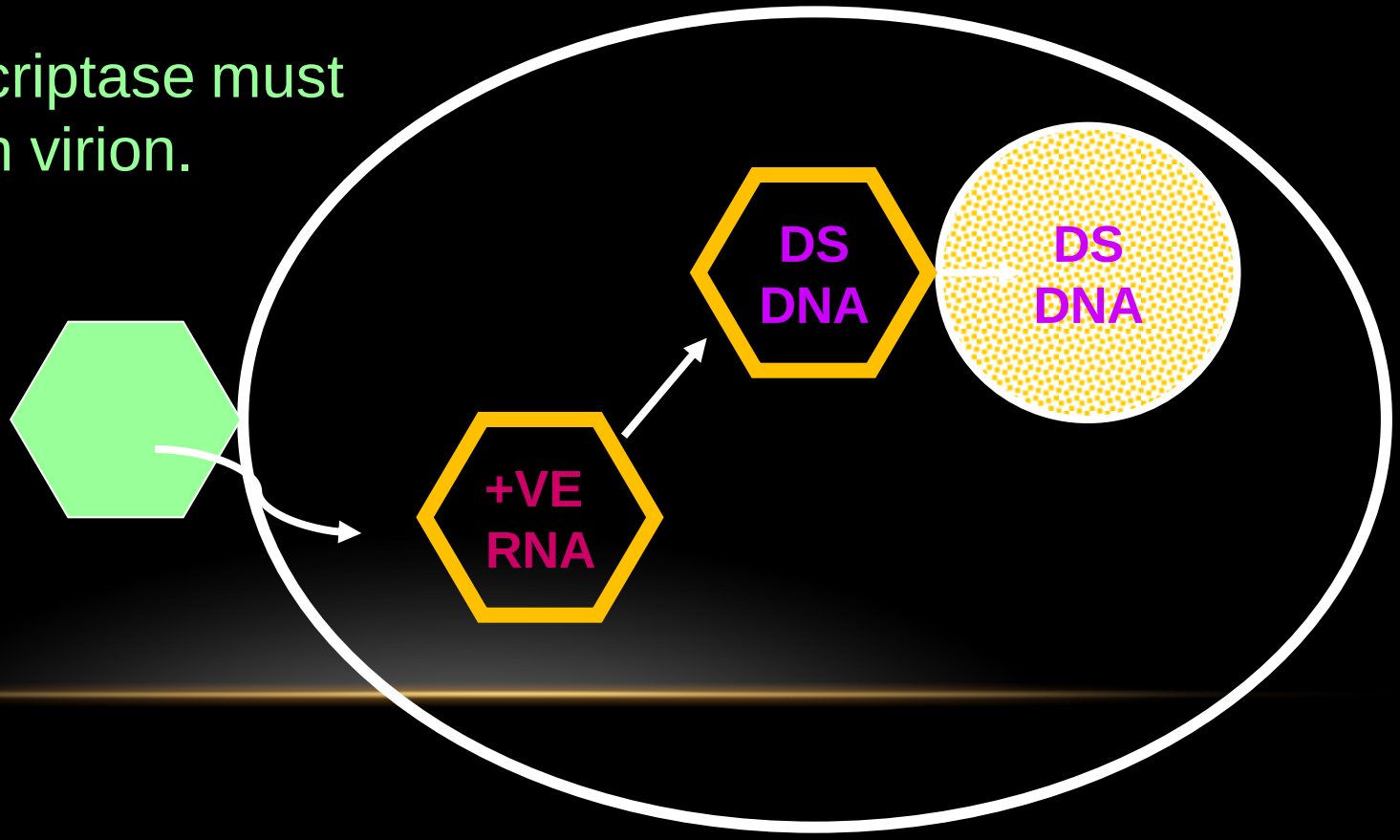


If used, RNA modifying enzymes are packaged in virion.

NEED TO MAKE MRNA

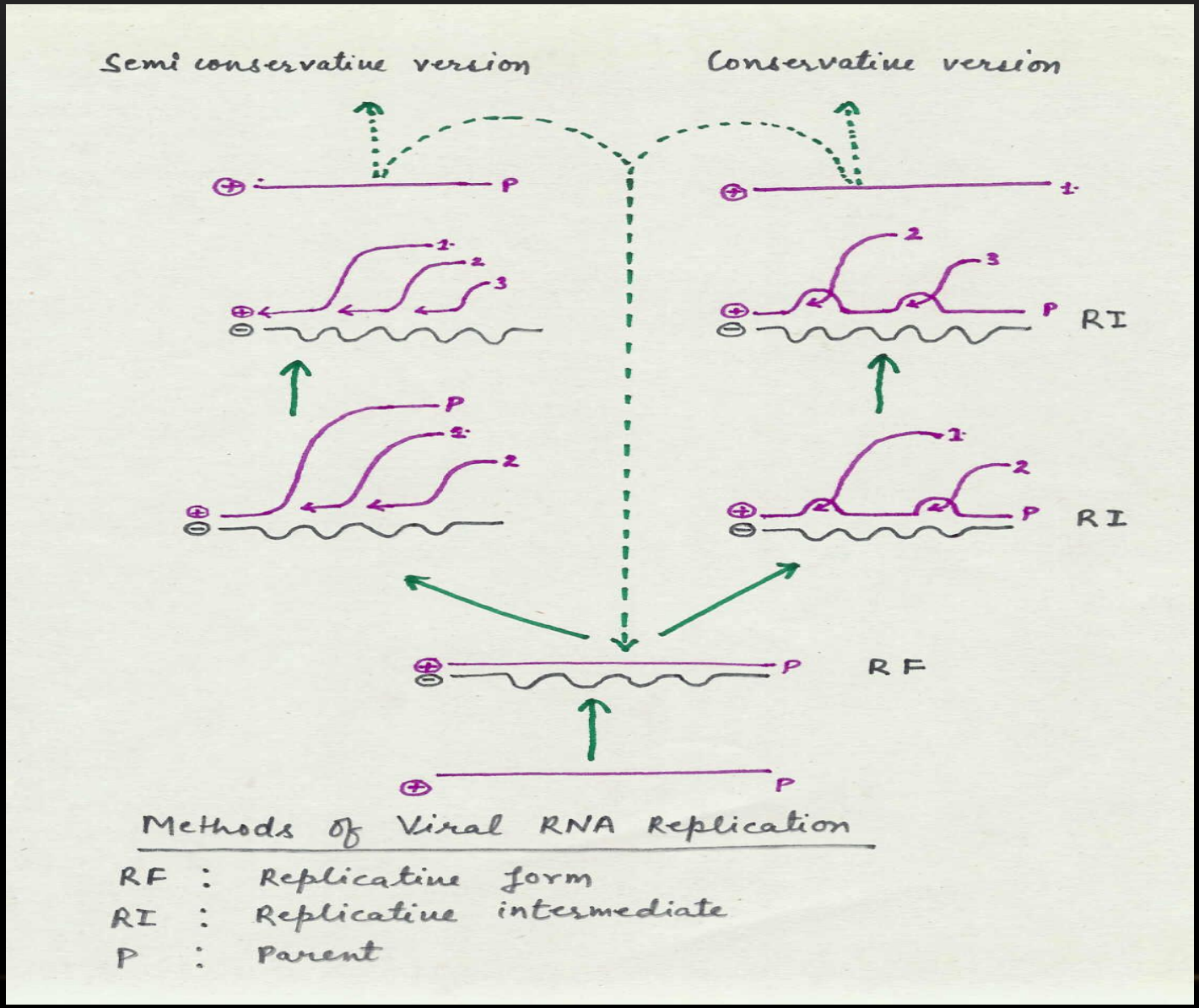
RETROVIRUSES

Reverse transcriptase must be packaged in virion.

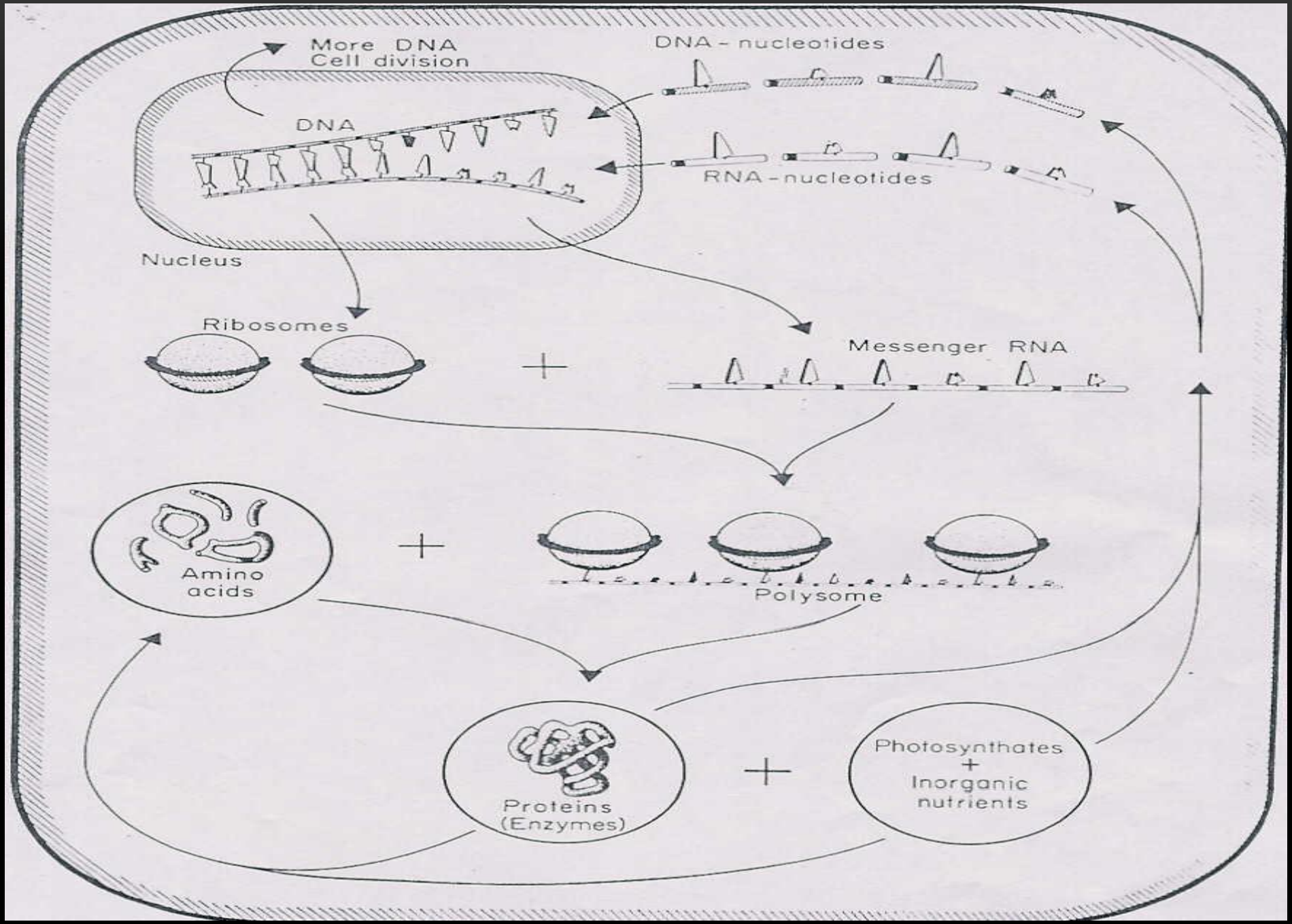


- Once viral genome enter the cell- replication start
- Its mediated by a **replicase** enzyme,
  - possibly coded partially by the viral genome and host genome.
- In the presence of polymerase, a –ve stand complementary to the genome +ve strand is formed.
- -ve strand serve as template for the synthesis of progeny or new +ve stand RNA by means of replicase enzyme forming a partially double stranded, partially single stranded structure, called Replicative intermediate (RI)

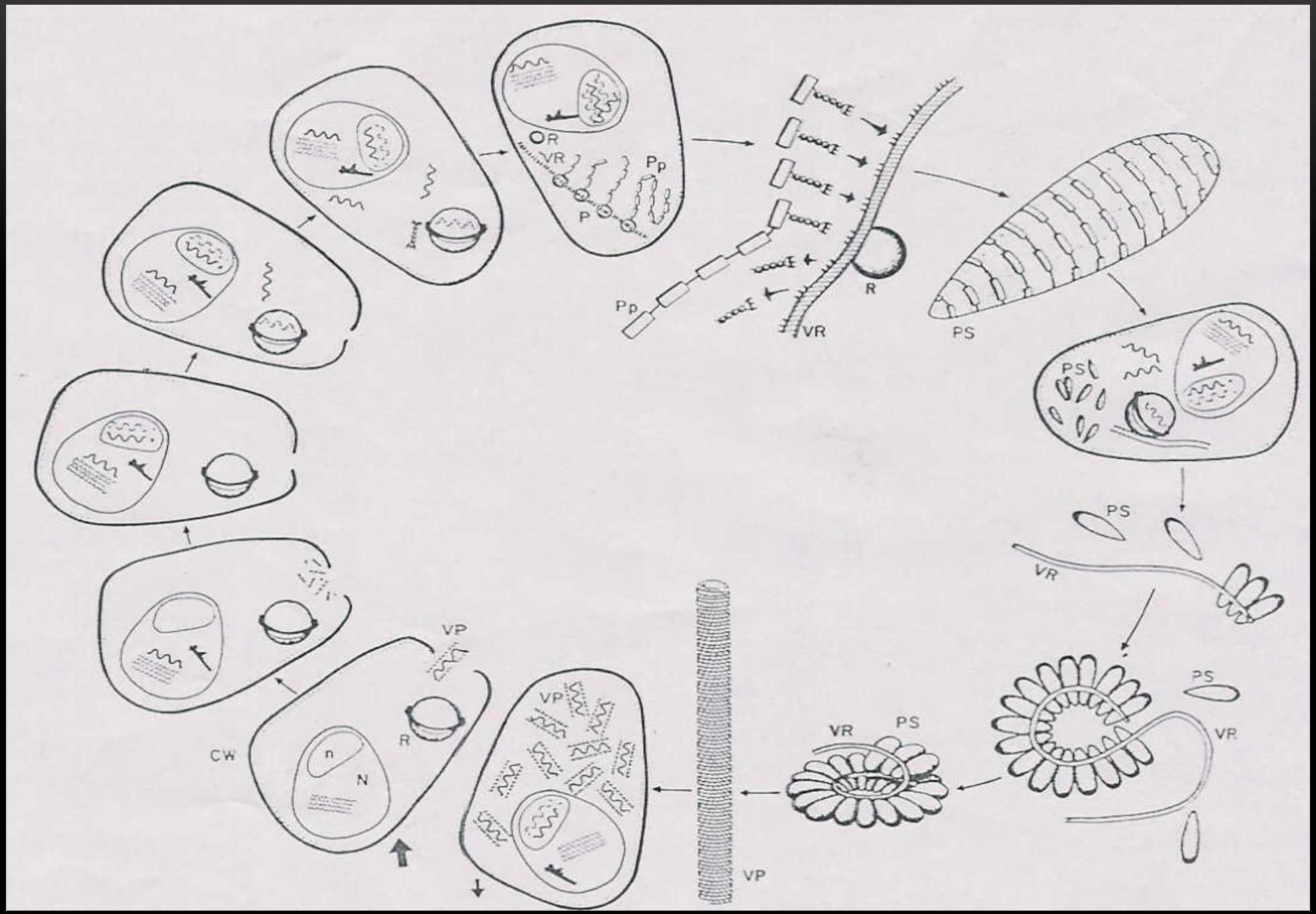
- A part of viral RNA form coat protein
- Synthesis of new RNA is from the 3’ to 5’ ends of the templates.
- Replication occurs in a replication complex that comprises of the template, newly synthesized RNA, the replicase and host factors.
- The viral RNA synthesizing systems have been shown to produce two kinds of RNA structures:
  - Replicative form (RF)- it is fully base paired ds and may represent RNA molecules that have seized the replication
  - Replication intermediate (RI) is only partly ds and contains several ss tails. This structure is closely related to that actually replicating the viral RNA.


Semi conservative version
Conservative version
P
1
2
3
RI
RF
Methods of Viral RNA Replication
RF : Replicative form
RI : Replicative intermediate
P : Parent

RI:



Protein Synthesis in Plant Cell

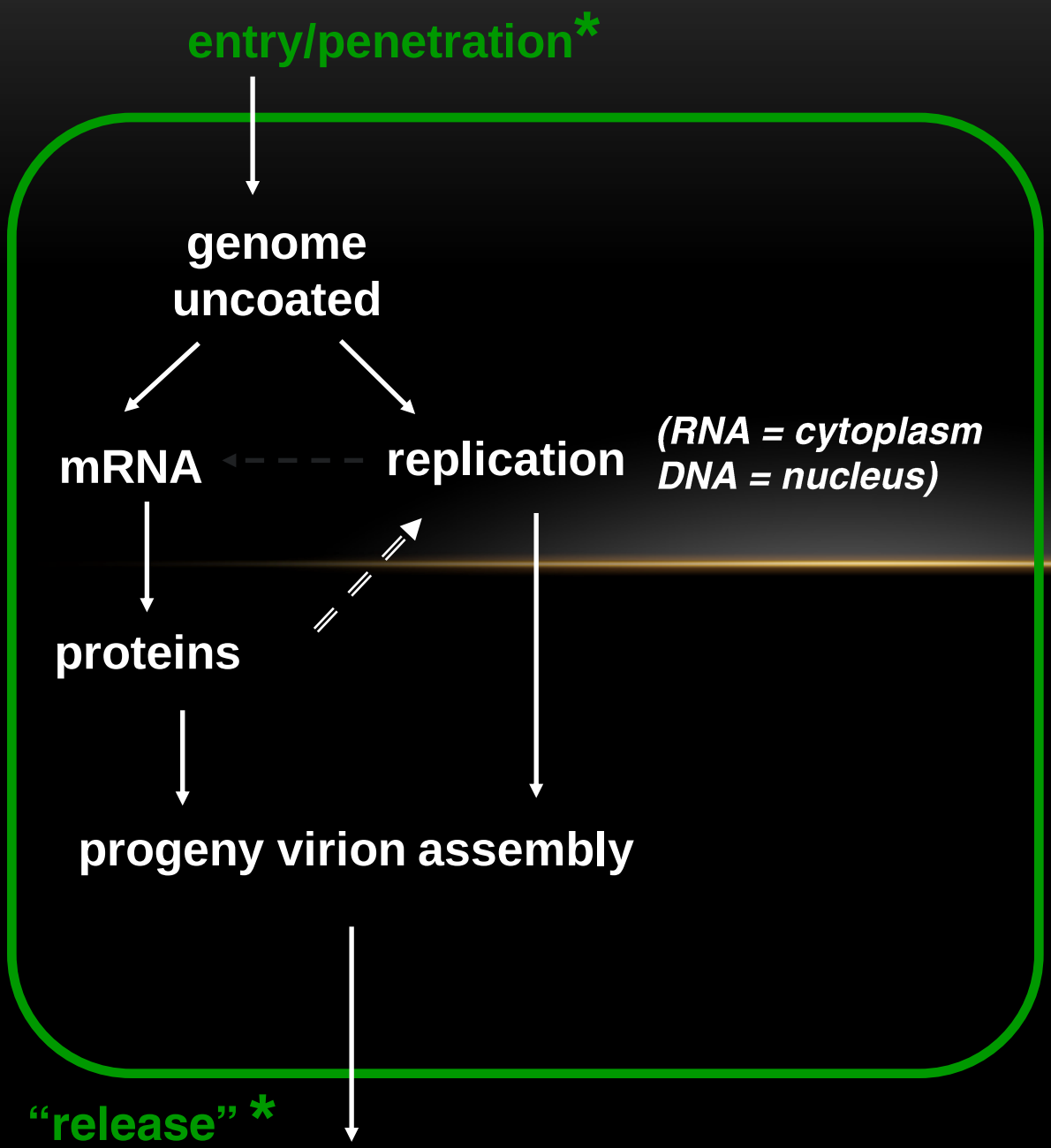


Infection Cycle of TMV

Sites for multiplication

- ✓ **Mostly nucleus and cytoplasm serve as sites for replication and assembly of virus.**
- ✓ **In some cases, chloroplast and other cell organelles perform the same function.**
- ✓ **In Potex, Caulimo, Gemini and Tobamo virus replication and assembly takes place in nucleus and from where it moves to cytoplasm.**
- ✓ **In Bromo, Poty, Nepo, Como viruses, replication and assembly takes place in cytoplasm.**
- ✓ **In Tymoviruses replication takes place in chloroplast.**

Plant Virus Life Cycle: Adaptations to the Cell Wall at Entry and Exit



Attachment/Penetration:
animal viruses bind to specific surface receptors;
Entry: fuse with or engulfed by the plasma membrane

Plant viruses: mechanical introduction initially. NO evidence attach to specific receptor sites on cell wall

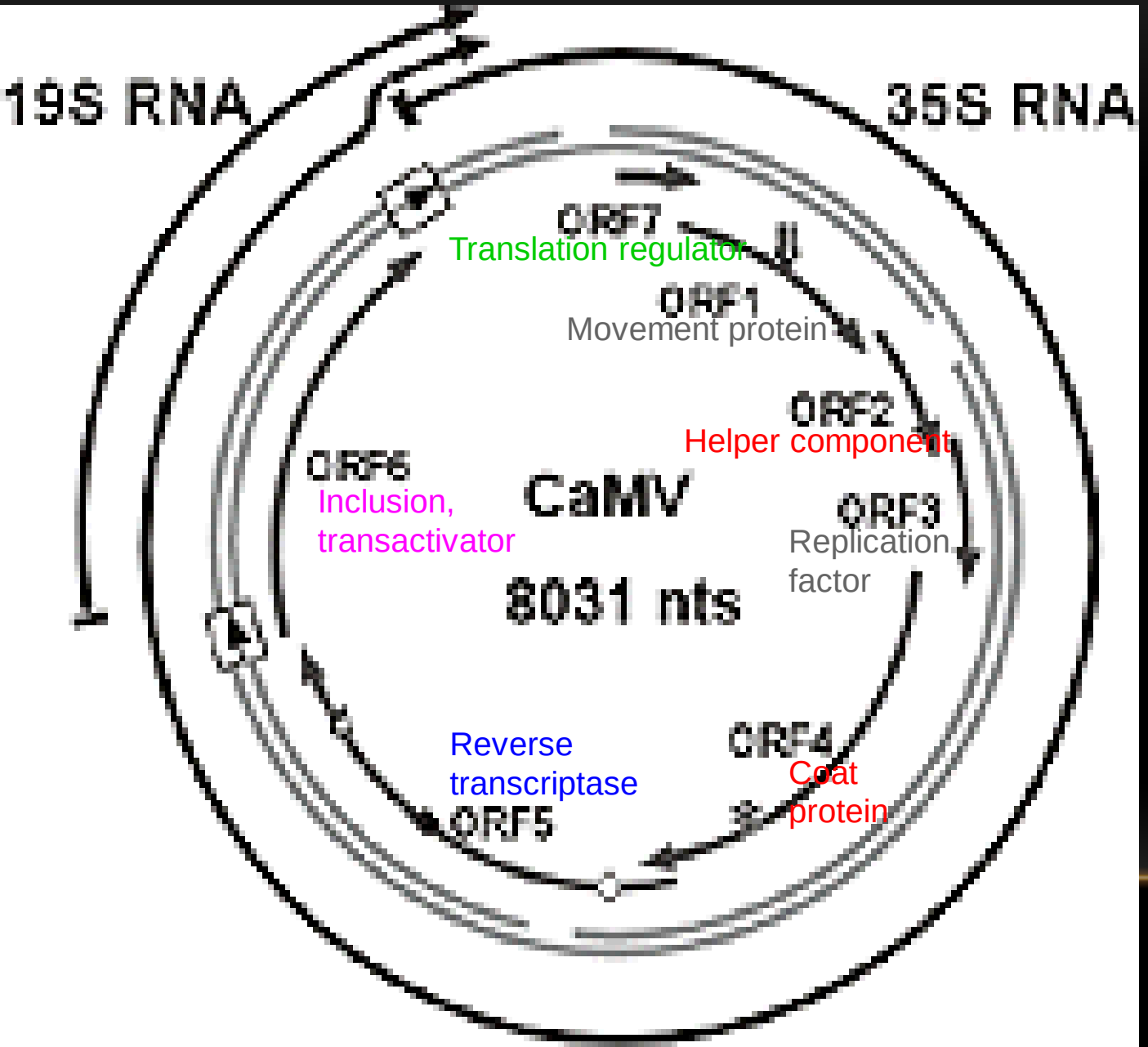
Transmission:
animal viruses: aerosols, break in skin, blood, sexual contact

plant viruses: insects, fungi, nematodes, abrasion, seeds, pollen, vegetative propagation

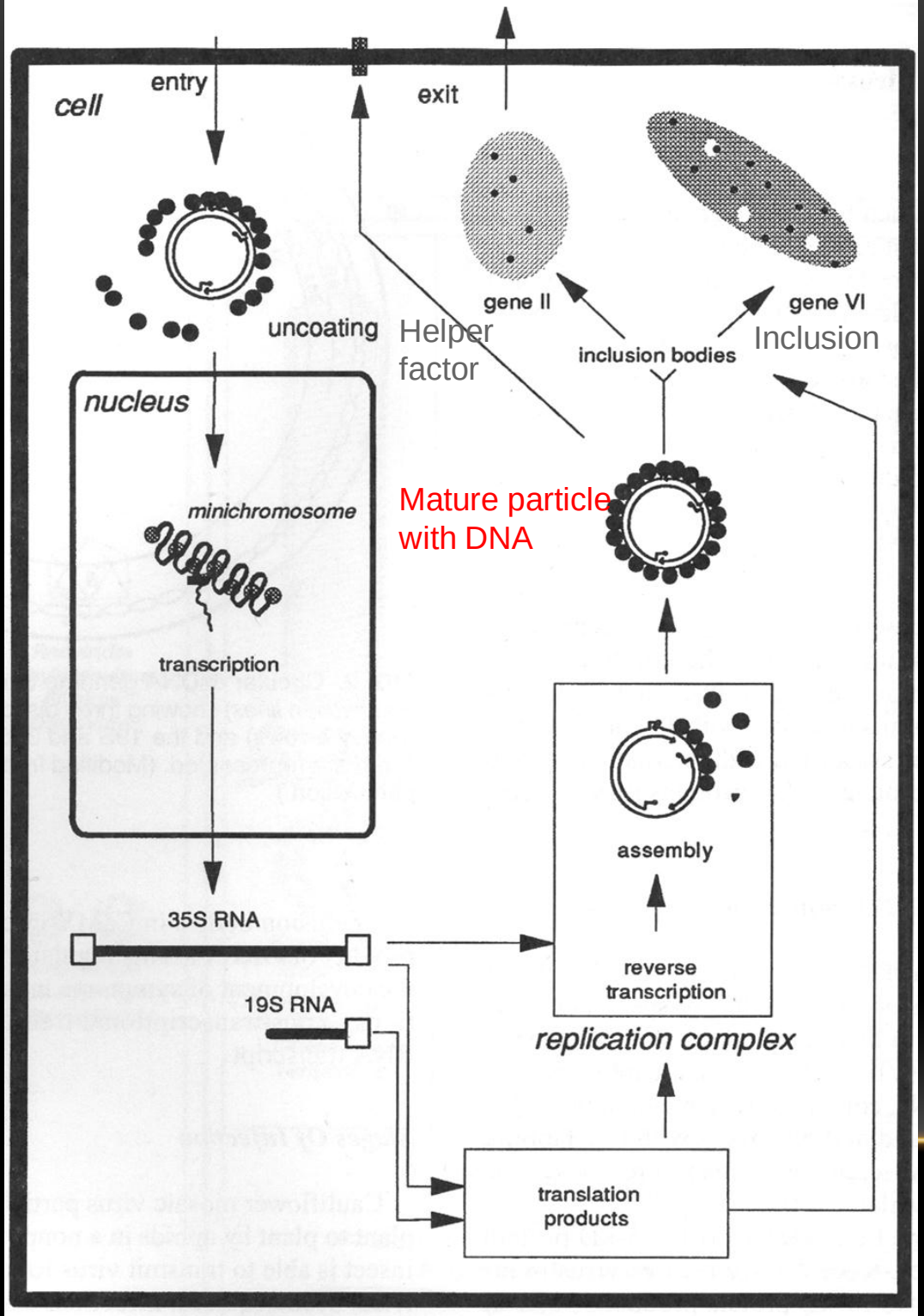
Release:
animal viruses lyse cells or bud through plasma membrane

plant viruses "channel" through wall (MPs) without lysis

CAULIFLOWER MOSAIC VIRUS GENOME STRUCTURE



- Seven ORFs on CaMV genome
- Translation of seven proteins from two transcripts
- ORF 2 is the only dispensable ORF
- ORFs 6 and 7 are involved in translation regulation
- Packaged genomic DNA has discontinuities on both strands
- Replication is from $tRNA^{met}$ primer



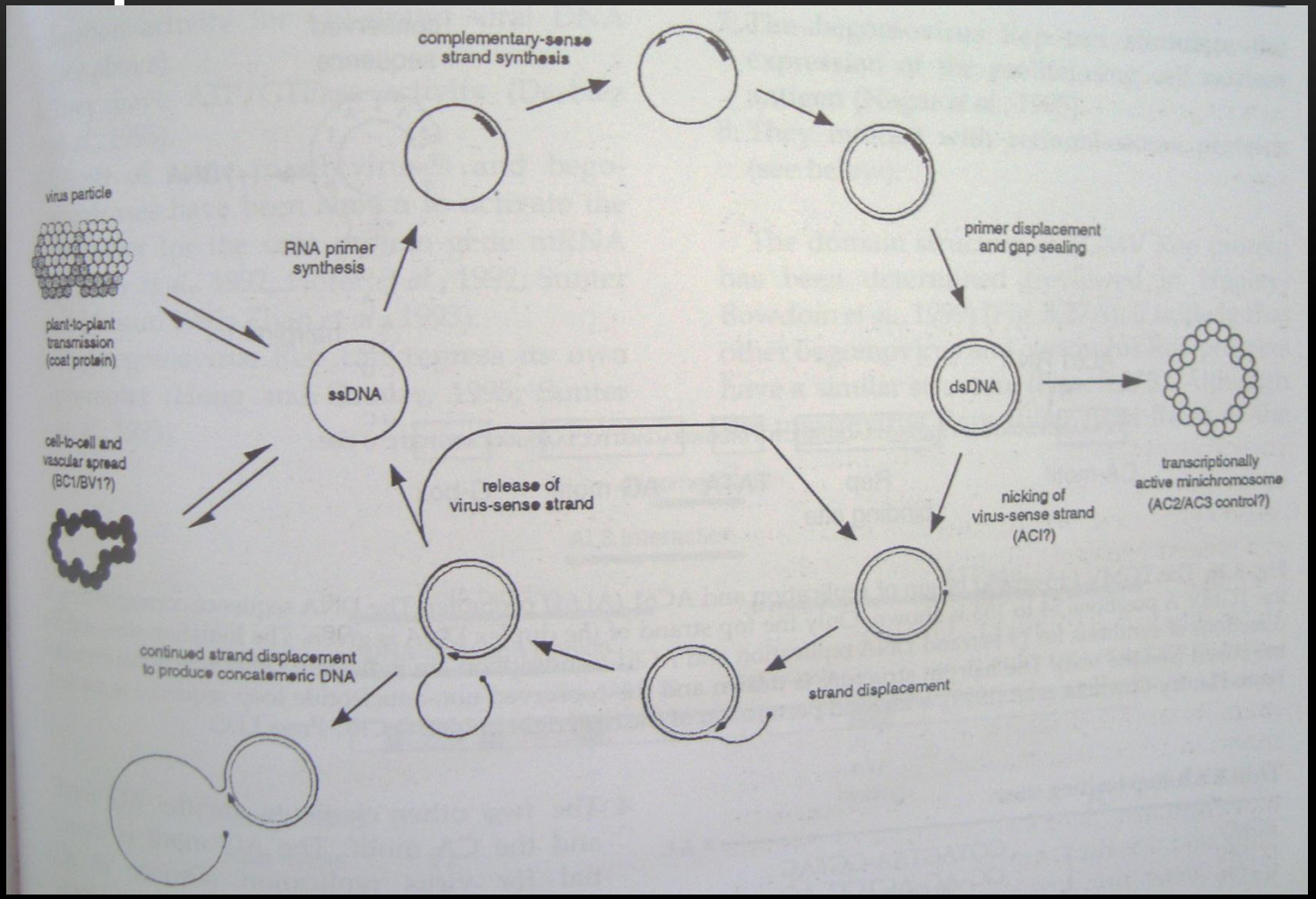
CAULIMOVIRUS LIFE CYCLE

- Virus enters plant cell, capsid protein is removed
- dsDNA enters nucleus; gaps closed; transcription to 35S and 19S RNAs
- In the cytoplasm, the 19S RNA is translated to produce protein that forms inclusion bodies
- Five ORFs are translated from 35S RNA by complex combination of strategies
- Other copies of 35S RNA are reverse transcribed and packaged into virions
- Particles exit cells through plasmodesmata or by aphids

Rolling Circle DNA Replication

- Common in plasmid or bacteriophage DNA and and the circular RNA genome of viroidse.g. geminiviruses
- Rolling circle DNA replication is initiated by **an initiator protein** encoded by the plasmid or bacteriophage DNA, which nicks one strand of the double-stranded, circular DNA molecule at a site called the **double-strand origin, or DSO**.
  - The initiator protein remains bound to the 5' phosphate end of the nicked strand, and the free 3' hydroxyl end is released to serve as a primer for DNA synthesis by DNA polymerase III.
  - Using the unnicked strand as a template, replication proceeds around the circular DNA molecule, displacing the nicked strand as single-stranded DNA. Displacement of the nicked strand is carried out by a host-encoded helicase called PcrA (plasmid copy reduced) in the presence of the plasmid replication initiation protein.
- Continued DNA synthesis can produce multiple single-stranded linear copies of the original DNA in a continuous head-to-tail series called a concatemer.
- These linear copies can be converted to double-stranded circular molecules through the following process:
  - First, the initiator protein makes another nick to terminate synthesis of the first (leading) strand. RNA polymerase and DNA polymerase III then replicate the single-stranded origin (SSO) DNA to make another double-stranded circle. DNA polymerase I removes the primer, replacing it with DNA, and DNA ligase joins the ends to make another molecule of double-stranded circular DNA.
  - Rolling circle replication has found wide uses in academic research and biotechnology, and has been

Ss DNA Virus Replication



- Involves rolling circle replication; It is a two step process
- 1. In the first step ss (+) strand is the template for the synthesis of (-) stand to generate a ds, replicative form (RF). This RF has two functions ie. It is the template for the transcription & is the template for the (+) strand synthesis generating free ssDNA.
- The priming of the (-) strand sunthesis us usually by an RNA molecule that is generated thorugh RNA polymerase or DNA primase activity. (+) stand sunthesis is primed by a site specifiic nick in the (+) stand of the RF

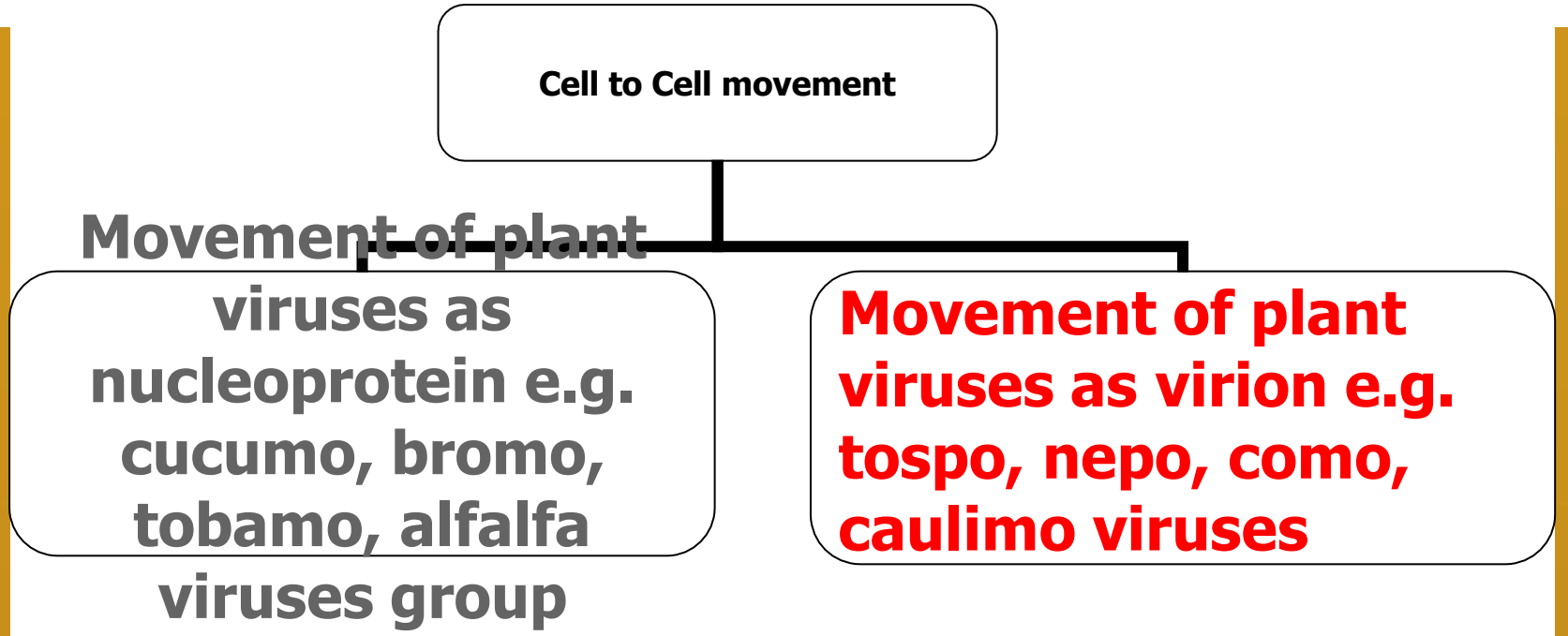
- Potyvirus has ss (+ve)RNA genome with a VPg protein covalently linked to its 5'end and encapsulated in about 2000 monomeric units of 32-35 kDa coat protein (Flores *et al.*,, 2003; Betty *et al.*, 2008).
- BCMV encodes 8 proteins whose functions have been characterized (Bos 1971, Urcuqui-Inchima *et al.*, 2001).
- The coding ORF is translated into one polyprotein of 340-370 Da which is processed into:
  - proteinase (Pro-1),
  - helper component proteinase (Hc-Pro),
  - proteinase (Pro-3),
  - a 6 kDa protein,
  - cylindrical inclusion (CI),
  - a second 6 kDa protein product,
  - nuclear inclusion a (NIa),
  - nuclear inclusion b (NIb) and
  - cp (Flores *et al.*, 2003).

A consensus potyvirus genome map

MOVEMENT OF PLANT VIRUSES

Movement of Plant Viruses

- Short distance movement
- Long distance movement



MOVEMENT OF VIRUS AS NUCLEOPROTEIN

Virus particle encode MP

↓

MP binds to viral RNA

↓

Binding unfolds viral RNA from random coil into linear rod shape structure

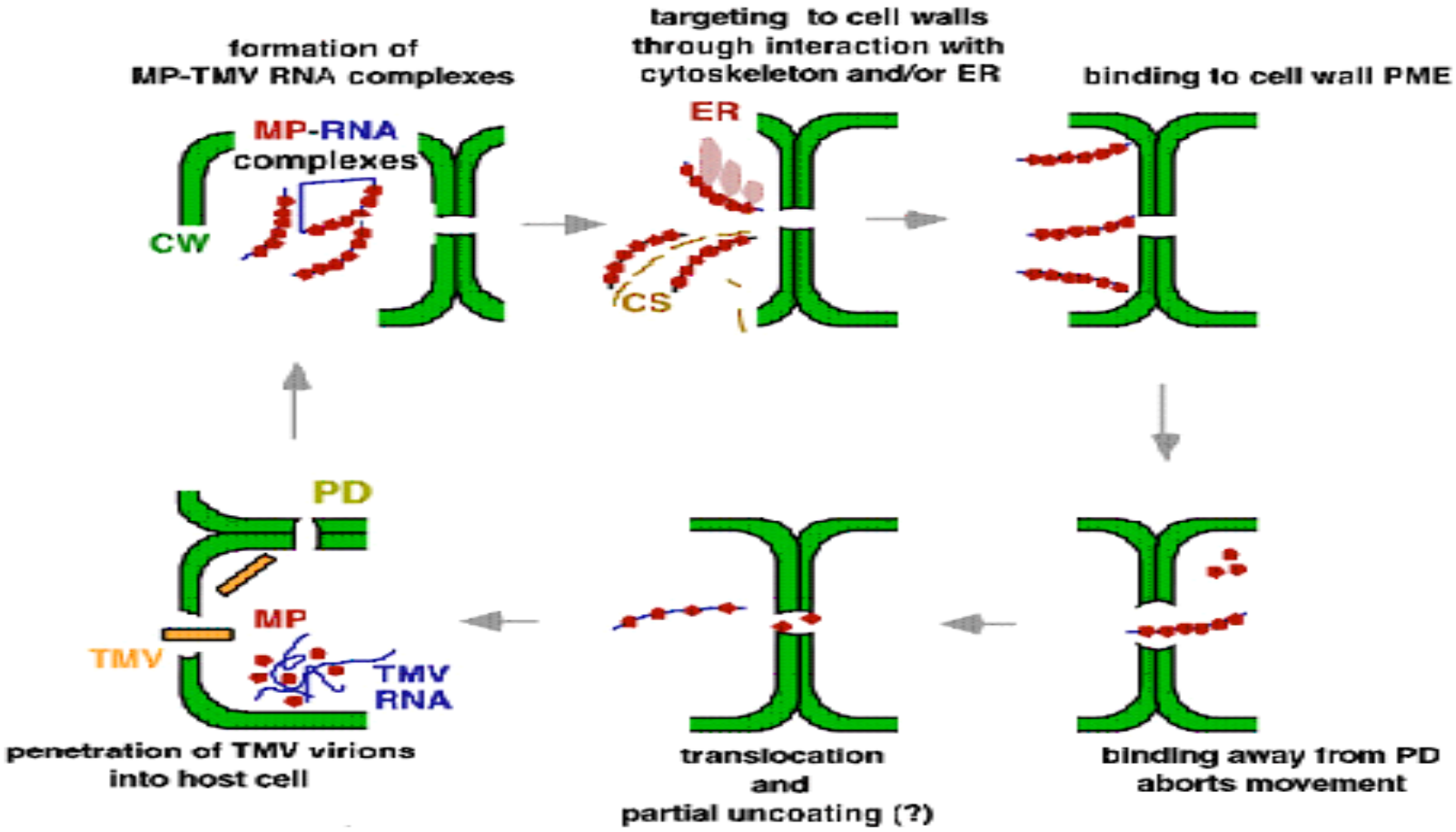
↓

MP RNA complex targeted to PD

↓

Through PD, movement to other cells

cell-to-cell movement of TMV



MOVEMENT OF VIRUS AS VRION

(Intercellular movement)

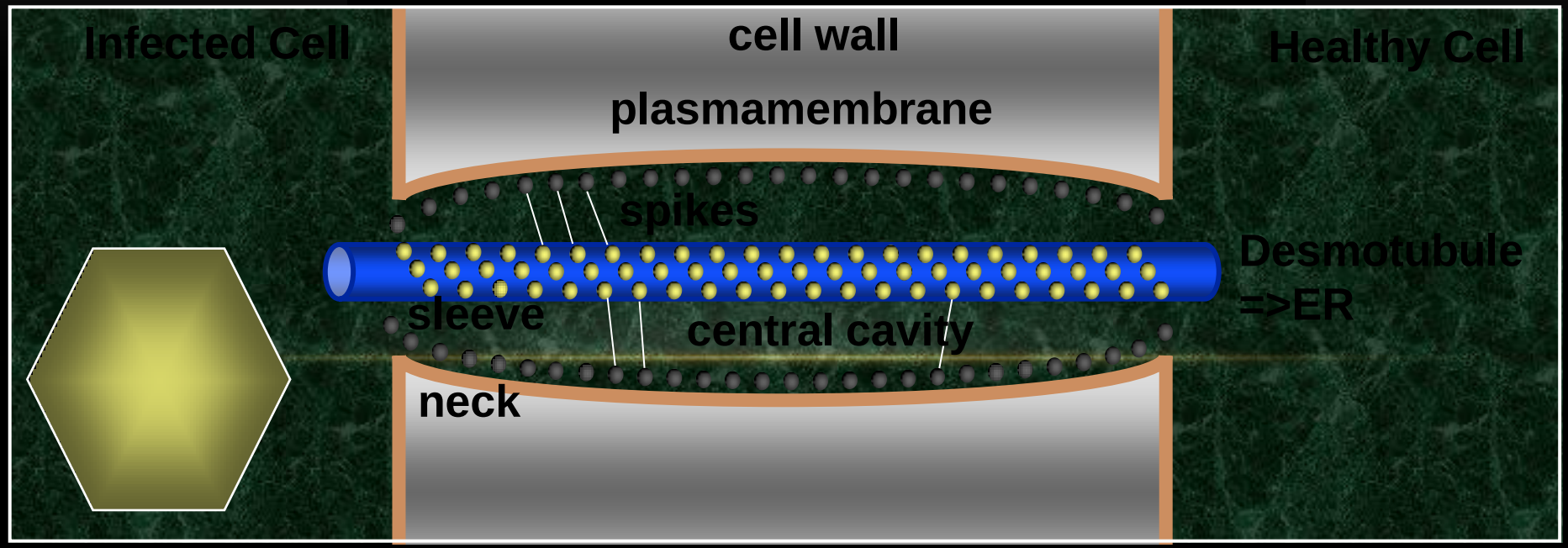
Formation of tubular structure in association with or through PD

Virus particle in linear form are observed inside these structure

Through cell wall, tubular structure move into neighbouring cells.

Deliver virus into that cell

Cell-to-cell Movement



Virus Movement Proteins (MPs) enable viral movement through plasmodesmata

Cell-to-cell movement strategies

Classification

TMV-like
Potyvirus-like
Closterovirus
Double Gene Block
Triple Gene Block
Geminivirus
Viroids
Tubule formers

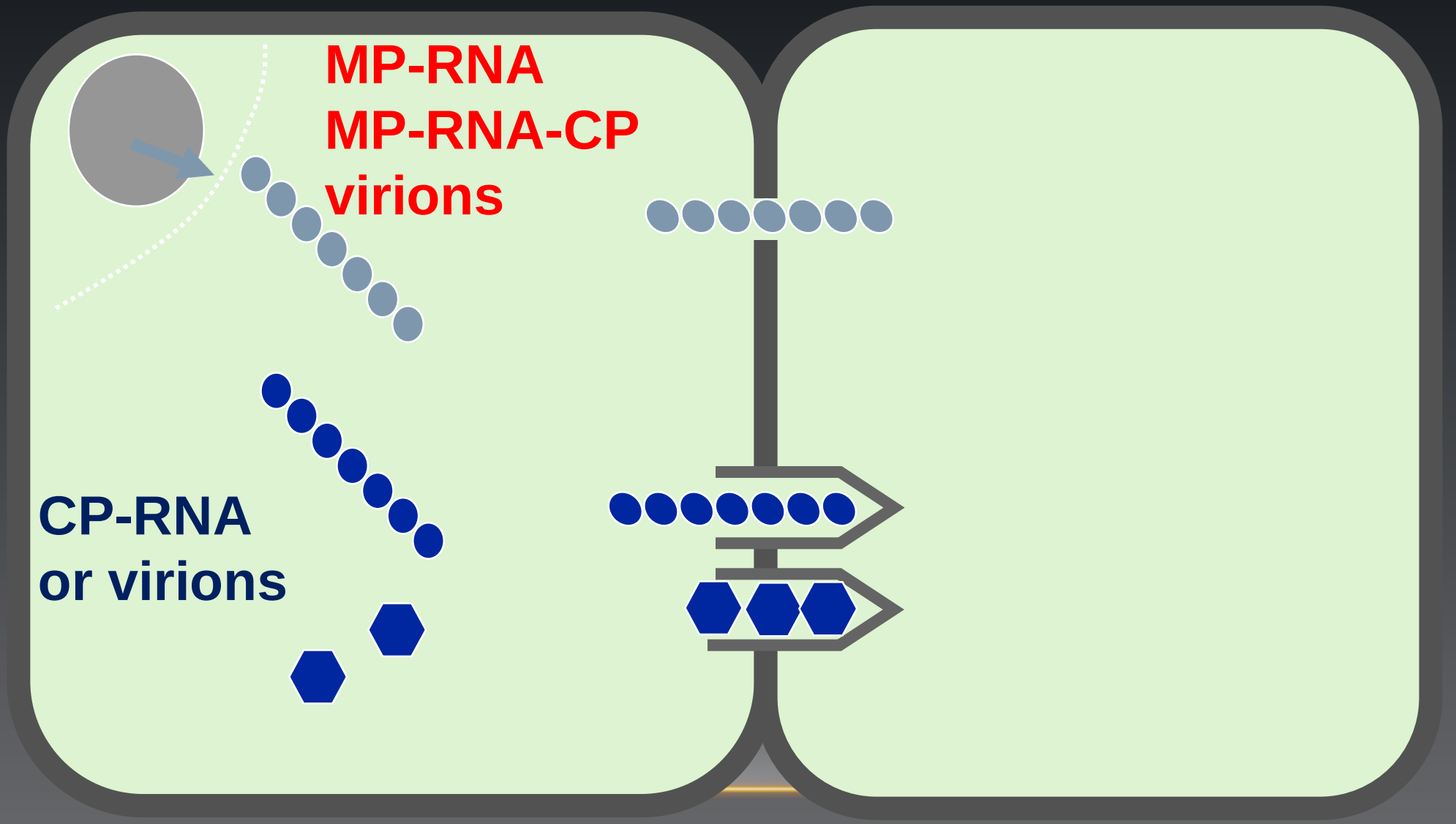
Different strategies can complement

Example:
AMV (*Bromoviridae*)
Needs MP plus CP

AMV MP can be complemented by:
1. CPMV MP (tubules, virion)
2. CMV MP (CP necessary)
3. TMV MP (no CP necessary)

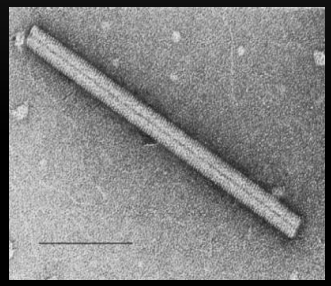
Sanchez-Navarro et al (2006) Virology 346:66

Different strategies



Extreme comparison

Tobacco mosaic virus **(TMV)**
Tomato spotted wilt virus **(TSWV)**

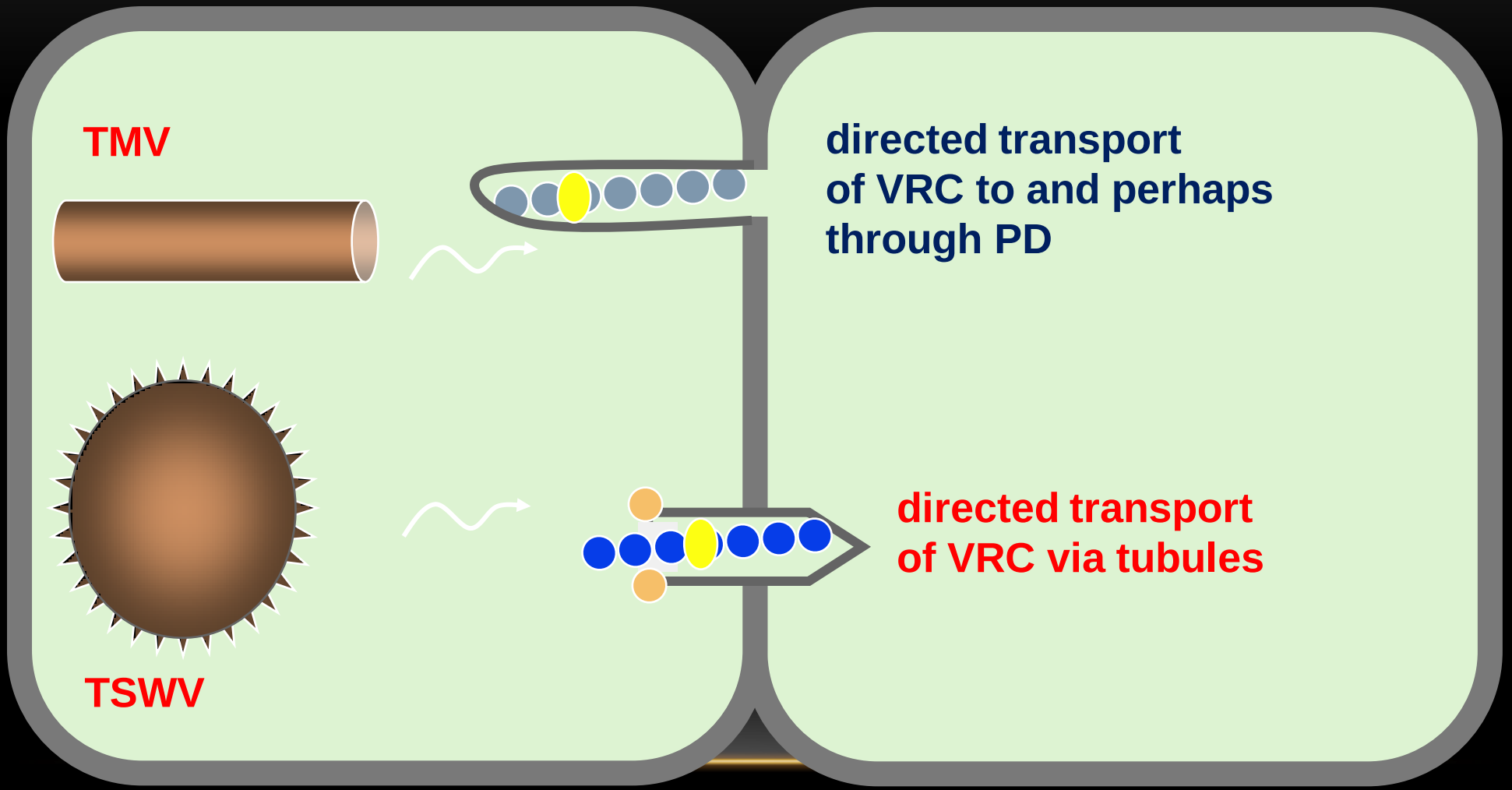


TMV, +RNA, no envelope
CP not required
RNP passes through PD

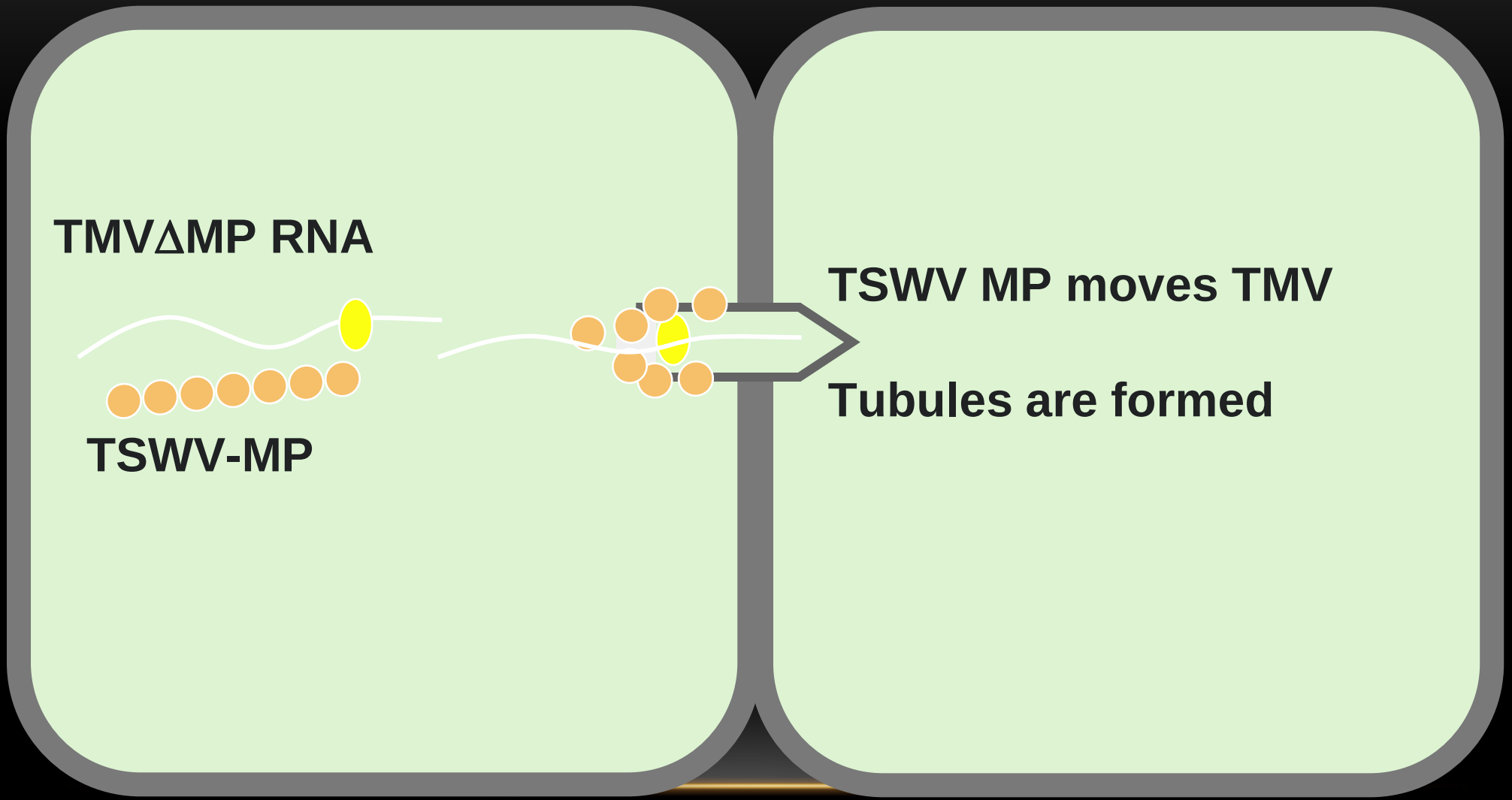
TSWV, +/-RNA, enveloped
CP (N) protein required
RNP in tubules that grow through PD

TSWV and TMV represent two extremes, or do they?

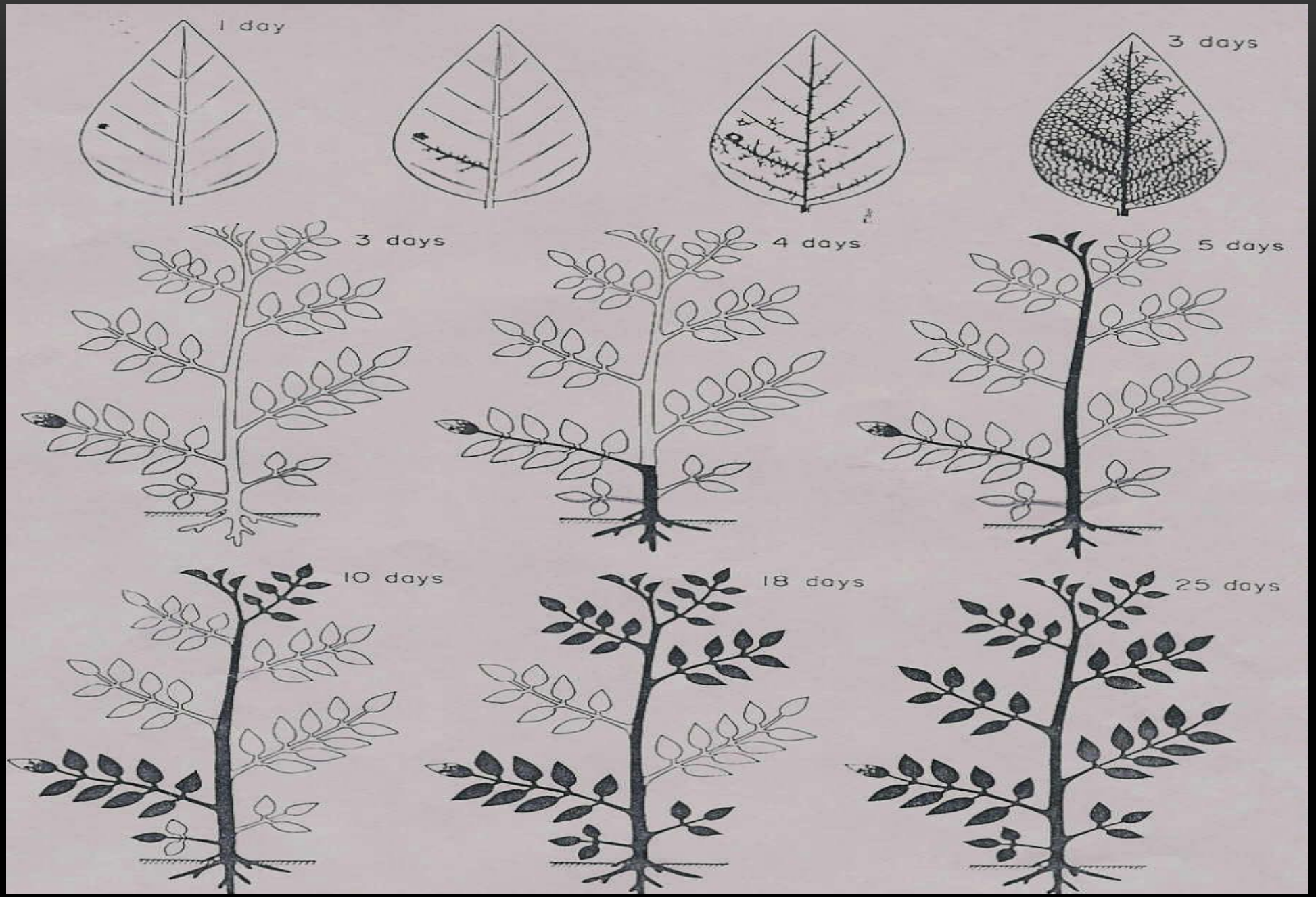
Transport of virus replication complexes (VRCs)



Complementation of TMV MP by TSWV MP



Lewandowski and Adkins (2005) Virology 342:26



Long distance Movement Within Plant

CONCLUSION

- Virus is a passive invader.
- Plant virus don’t have specific attachment sites
- Virus uncoating is a first step in infection cycle.
- Plant virus make use of host cell machinery for nucleic acid and protein synthesis.
- Cell to cell movement and long distance movement are the two ways of virus spread within plant.
- Plant-virus interaction depends upon genotype of host i.e. Immune, Resistant, Tolerant and susceptible.

ACKNOWLEDGEMENTS

- I gratefully acknowledge the use of text book “Matthews Plant Virology” by Roger Hull.
- I acknowledge the scientists who spent valuable time in generating information on various aspects of plant Virology and displayed the same on internet for use by students, teachers and researchers

PN Sharma