COMPARATIVE PLANT VIROLOGY

SECOND EDITION

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CHAPTER

7

Expression of Viral Genomes

Viral genomes are expressed via mRNAs. Eukaryotic cells pose constraints on how the information on an mRNA is expressed. Viruses have a variety of ways of overcoming these constraints.

OUTLINE

I. STAGES IN VIRUS INFECTION CYCLE

The infection cycle of a virus, be it of a plant, vertebrate, invertebrate, or bacterium, has seven stages (see Figure 7.1):

1. Virus initial entry into the cell (discussed in Chapter 12).
2. Genome uncoating (discussed in this chapter).
3. Production of mRNAs. As will be shown in this chapter, the route used for the production of mRNAs depends on the nature of the viral genome.
4. Translation of the viral genetic information from the mRNAs. Some of this information is expressed early and some late in the infection cycle, depending on when the product is required.

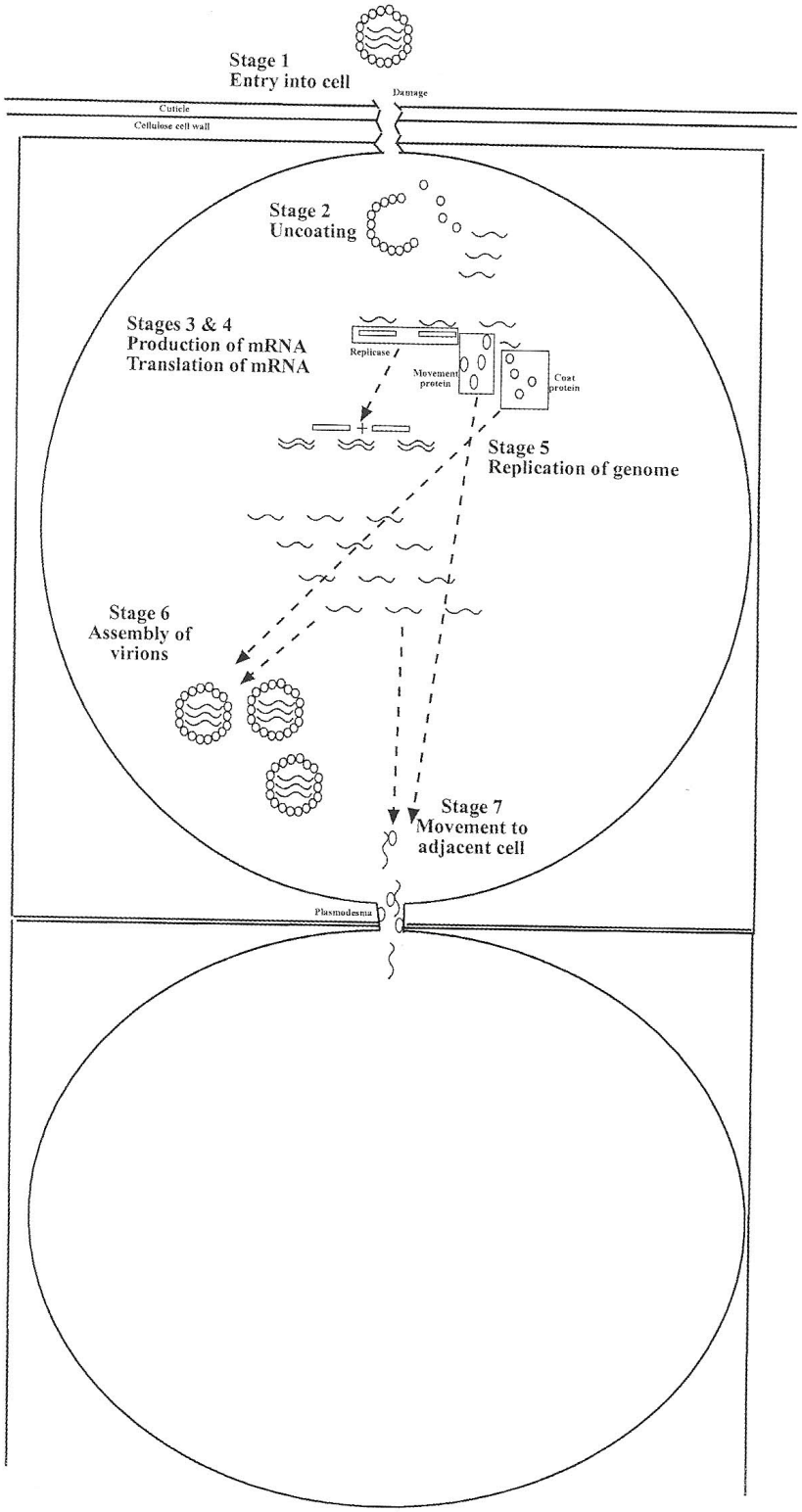

FIGURE 7.1 The seven stages of a virus infection cycle. The square box represents the plant cell wall and the circle within it, the plasma-membrane.

5. Replication of the viral genome using at least some of the factors expressed from the viral genome (discussed in Chapter 8).
6. Assembly of the progeny virions (discussed in Chapter 5).
7. Release of virus from initially infected cell and infection of adjacent cells (discussed in Chapter 9).

However, we must bear in mind that these seven stages are not completely separate and sequential and that they are closely integrated and coordinated.

II. VIRUS ENTRY AND UNCOATING

A. Virus Entry

As we will see in Chapter 12, plant viruses require damage to the cuticle and cell wall to be able to enter a plant cell. There is no evidence for a specific entry mechanism such as plasma membrane receptor sites or endocytotic uptake as with viruses of vertebrates and invertebrates, and it is generally considered that entry is accomplished by "brute force".

B. Uncoating

Once in the initially infected cell, the virus particle has to be uncoated to release the genomic nucleic acid. There is a dichotomy in the structural stabilisation of viruses in that the particles have to be stable enough to protect the viral genome when being transported outside the host but must be able to present the genome to the cellular milieu for the first stages in replication. The uncoating process depends on the chemical bonds that stabilise the virus particles (see Chapter 5). This has been studied for the rod-shaped *Tobacco mosaic virus* (TMV) and for several isometric viruses.

1. Uncoating of TMV

Using TMV radioactively labeled in the protein or the RNA or in both components, the following has been observed:

1. Within a few minutes of inoculation, about 10 percent of the RNA may be released from the virus retained on the leaf.
2. Much of the RNA is degraded, but some are still full-length RNA molecules.
3. The early stages of uncoating of the particles do not appear to depend on preexisting or induced enzymes.
4. The process is not host specific, at least in the early stages.

However, there is a fundamental difficulty with all such experiments. Concentrated inocula must be used to provide sufficient virus for analysis, but this means that large numbers of virus particles enter cells rapidly. Thus, it is impossible to know which among these particles actually establish an infection.

To initiate infection, TMV RNA must be uncoated, at least to the extent of allowing the first ORF to be translated. By various *in vitro* experiments, it was shown that TMV is uncoated by a process termed *cotranslational disassembly* (Box 7.1).

2. Uncoating of Brome Mosaic Virus and Southern Bean Mosaic Virus

The isometric particles of *Brome mosaic virus* (BMV) and *Southern bean mosaic virus* (SBMV) are stabilised by carboxyl-carboxylate bonds and by protein-RNA interactions; SBMV particles are also stabilised by Ca^{2+} (see Chapter 5). Above pH 7, the carboxyl-carboxylate bonds protonate, and after the removal of Ca^{2+} from SBMV, the particles of both viruses swell, being just stabilised by protein-RNA interactions. These swollen particles can be uncoated by

BOX 7.1

COTRANSLATIONAL DISASSEMBLY OF TMV

The first event in cotranslational disassembly of TMV is that the structure of the virion relaxes so the 5′ terminus of the RNA is accessible to a ribosome. The 68 nucleotide 5′ leader sequence, which lacks G residues, interacts more weakly with coat protein subunits than do other regions of the genome. As discussed in Chapter 5, TMV particles are stabilised by carboxylate interactions, which become protonated at slightly alkaline pHs. This allows a ribosome to attach to the 5′ leader sequence and then to move down the RNA, displacing coat protein subunits as it moves. The ribosome-partially-stripped-rod complexes are termed *striposomes* (Figure A), which have been found both *in vivo* and *in vitro*.

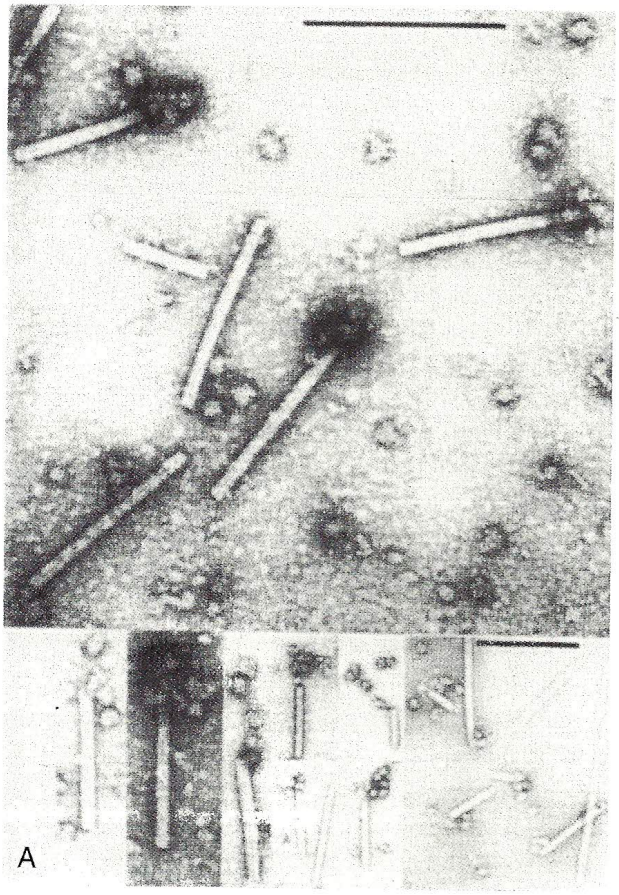

Fig.A. Electron micrographs of *striposome* complexes showing ribosomes clustered around one end (the 5′ end) of TMV rods. [This article was published in *Virology*, **137,** T.M. Wilson, Cotranslational disassembly of *tobacco mosaic virus in vitro*, pp. 255–265, Copyright Elsevier (1984).]

Having initiated translation, ribosomes proceed along TMV RNA, translating the 5′ ORF and the 126/183 kDa replicase protein (see Profile 14 in the Appendix for TMV genome map) and displacing

(continued)

BOX 7.1 *(continued)*

coat protein subunits. When the ribosomes reach the stop codon of the 126/183 kDa ORF, they disengage. This raises the question of how the 3′ quarter of the particle is disassembled. It is considered that the replicase performs this task in a 3′→5′ direction in synthesising the (–)-strand replication intermediate in a process termed *coreplicational disassembly* from the 3′ end.

Thus, TMV is uncoated in a bidirectional manner, using the cotranslational mechanism for the 5′→3′ direction, yielding the replicase that disassembles the rest of the particle in the 3′→5′ direction, showing that disassembly and replication are coupled processes. The process happens rapidly with the whole capsid uncoated within about 30 minutes (Figure B).

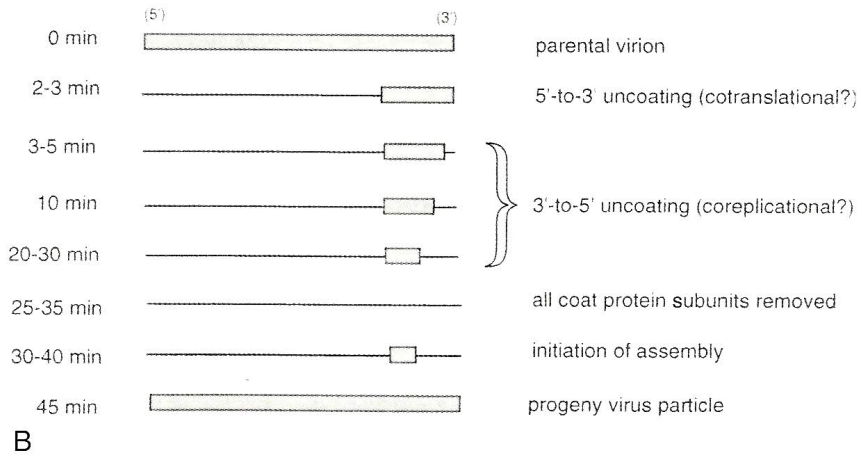

Fig.B. Time scale of bidirectional disassembly and assembly of TMV particle *in vivo*. [From Wu and Shaw (1996), *Proc. Natl. Acad. Sci. USA*, **93**, 2981–2984, Copyright (1996) National Academy of Sciences, U.S.A.]

cotranslational disassembly *in vitro* translation. However, mutants of another bromovirus that did not swell under alkaline conditions showed that swelling was not necessarily required for cotranslational disassembly, leading to the suggestion that there is a pH-dependent structural transition in the virion, other than swelling, which enables the RNA to be accessible to the translation system. The proposed model, which is similar to those of some vertebrate and insect viruses, postulates that the N termini of the five subunits in the pentameric capsomere undergo a major structural transition from the interior to the exterior of the virion. This provides a channel through which the RNA passes to be accessible for translation. However, the 5′ end of the RNA must be released, which suggests that it is located in association with a pentameric capsomere.

3. *Uncoating of Turnip Yellow Mosaic Virus*

The isometric particles of *Turnip yellow mosaic virus* (TYMV) do not cotranslationally disassemble in the *in vitro* translation system

just described for BMV and SBMV. *In vitro* studies show that under various nonphysiological conditions, such as pH 11.5 or freezing and thawing, the RNA can escape from TYMV particles without disintegration of the protein shell. When Chinese cabbage leaves are inoculated with TYMV, a significant proportion of the inoculum is uncoated after two minutes, with empty virus particles and low-MW protein being formed following RNA release. At least 80 to 90 percent of this uncoating takes place in the epidermis. This uncoating process is not confined to known hosts of TYMV.

4. *Uncoating Other Plant Viruses*

The requirements in the first stages of infection with other types of genomes are different to those of the (+)-sense ssRNA viruses. Viruses with dsRNA or (–)-sense ssRNA have to transcribe their genome to give mRNA. These viruses carry the viral RNA-dependent RNA polymerase (RdRp) in the virus particle and, presumably, transcription is an early event. It is not known if this occurs within the virus particle, possibly in a relaxed structure, or if the viral genome is released into the cell. However, it is most likely that this process takes place in an environment that is protected from cellular nucleases and that it is coupled to translation of the mRNA.

The dsDNA genomes of members of the *Caulimoviridae* must be transported to the nucleus, where they are transcribed to mRNA by the host RNA-dependent RNA polymerase (see later in this chapter). The coat protein of *Cauliflower mosaic virus* (CaMV) has a nuclear localisation signal that will presumably target the particle into the nucleus. Particles of some caulimoviruses and badnaviruses are particularly stable, capable of resisting phenol, and nothing is known about how they disassemble.

The ssDNA genomes of members of the *Geminiviridae* also have to be transported to the nucleus so they can be replicated before being transcribed to give mRNAs. Nuclear localisation signals have been recognised in some geminiviral proteins, but nothing is known about how the particles uncoat.

III. INITIAL TRANSLATION OF VIRAL GENOME

Viral genomes are expressed from mRNAs that are either the nucleic acid (+)-sense ssRNA viruses or transcripts from the (–)-sense or dsRNA, or from ds or ss DNA viruses. Baltimore (1971) pointed out that the expression of all viral genomes, be they RNA or DNA, ss or ds, (+)- or (–)-sense, converges on the mRNA stage (Figure 7.2). As we will see later in this chapter, expression of the viral mRNA faces various constraints imposed by the eukaryotic translation system.

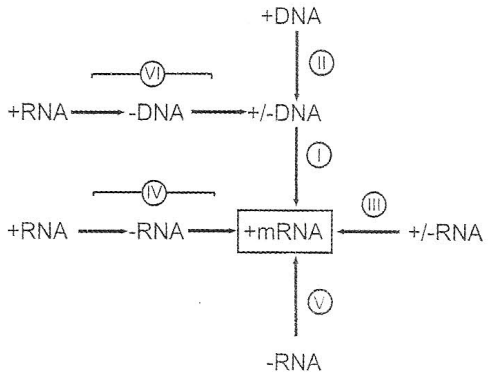

FIGURE 7.2 Routing of viral genome expression through mRNA. Route I is transcription of dsDNA usually by the host DNA-dependent RNA polymerase; route II is transcription of ssDNA to give a dsDNA template for I (e.g., geminiviruses); route III is transcription of dsRNA, usually by virus-coded RdRp (e.g., reoviruses); route IV is replication of (+)-strand RNA via a (–)-strand template by virus-coded RdRp—the viral (+) strand is often the template for early translation (the (+)-strand RNA viruses); route V is transcription of (–)-strand RNA viral genome by virus-coded RdRp (e.g., tospoviruses); route VI is reverse transcription of the RNA stage of retro- and pararetroviruses leading to a dsDNA template for mRNA transcription (for pararetroviruses the input viral dsDNA can be the template. [From Baltimore (1971).]