1 INTRODUCTION

I. POSITIVE-SENSE RNA VIRUSES

RNA is labile; still it carries genetic information and multiplies efficiently. About 80% of viruses possess single-stranded RNA genome of positive polarity (positive-strand/ positive-sense/plus-sense/plus-strand/messenger-sense) so that it is understandable that a great majority of studies on replication of viruses pertain to these viruses. Positive-sense RNA viruses encompass over one-third of all virus genera and include numerous (plant, animal, and human) pathogens, such as the *Severe acute respiratory syndrome coronavirus* (SARS), *Hepatitis C virus*, and many of the viruses on the US Health and Human Services Department Select List of potential bioterrorism agents (Ahlquist *et al.*, 2003). Early work on computer-assisted comparative studies for understanding the relationships within these positive-strand RNA viruses (Franssen *et al*., 1984; Haseloff *et al*., 1984; Argos *et al*., 1984; Kamer and Argos, 1984; Ahlquist *et al*., 1985) demonstrated the presence of non-trivial links between these viruses of plants and animals (Koonin and Dolja, 1993). In fact, this was the least expected outcome of the comparative analysis of their RNA genomes (Goldbach, 1987). Many of the characteristics of positive-sense RNA viruses of animals and plants, particularly those concerned with RNA replication, are the same or nearly the same so that these viruses are now regarded to form a single unit.

Vast differences exist in morphology of virus particles, organization of genomic RNAs, host ranges, symptoms, and some other characters of positive-sense RNA viruses of plants (like *Brome mosaic virus*, *Cucumber mosaic virus*, *Tobacco mosaic virus* and others) and animals (like *Sindbis virus* and *Poliovirus*). Still these viruses exhibit striking similarities in replication strategies (Pogue *et al.*, 1994). For example, amino acid sequences of their virus-encoded RNA-dependent RNA polymerases (RdRps) and several other functional domains within other virus-encoded non-structural proteins (helicases, nucleoside triphosphate motifs, proteinases) of these viruses are conserved (Koonin, 1991a, 1991b; Koonin and Dolja, 1993; Dougherty and Semler, 1993; Zanotto *et al*., 1996; Buck, 1996; Kadaré and Haenni, 1997). The above similarities clearly establish that these completely different plant and animal viruses and virus groups have identical RNA replication mechanisms although the nature of their replication proteins (like amino acid sequences of viral RdRps) and their interacting host proteins are different. The similarities between these positive-sense animal and plant viruses extend even to their having related genes and occasionally even possessing identical gene organization and gene expression. Thus, the replication of plus-sense viral RNA *per se* or its different stages has been reviewed innumerable times in viruses in general and/or in different groups of viruses since the sixties of the last century (Hofschneider and Husen, 1968; Montagnier, 1968) till date (White and

Nagy, 2004; and many other references concerning plant viruses mentioned in text at relevant places in different chapters).

Some of the main principles of evolution of positive-sense RNA viruses, as formulated by Koonin and Dolja (1993) are: RNA viruses evolve rapidly so that only very important functional motifs are conserved in a wide range of virus groups; the universal building blocks of positive-sense RNA viruses are the genes for RdRp and capsid protein, out of which only RdRp sequence contains universal motifs that are conserved in all positive-sense RNA viruses with known genome sequences; evolution of positive-sense RNA viruses is shaped by two opposing trends - conservation of distinct arrays of genes mainly those encoding viral replication proteins and recombinational shuffling of genes and gene blocks; widespread recombination, even among distantly related viruses, has made it impossible to depict evolutionary history of positive-strand RNA viruses as a single phylogenetic tree; and correlation between virus phylogeny and strategy of genome replication and expression is only limited - suggesting that fundamental expression and replication mechanisms could have evolved more than once.

The positive-sense RNA viruses exhibit great genetic compactness so much so that individual nucleotides often perform multiple functions during their life cycle. The virus genomic RNA of positive-sense RNA viruses nearly always serves two essential functions at the start of viral replication cycle. It acts at two independent levels: it acts as messenger RNA encoding the various virus-specific proteins [coat protein, replication proteins (polymerase, helicase, proteinase), movement protein, and still other proteins involved in viral RNA replication]. Then, it also acts as template for transcription into minus-strand RNA, which is the starting point of RNA replication process. But, there is no interference between these two functions of a single genomic RNA molecule. This means that there is no clash between the ribosomes reading the RNA from its 5`- to 3`-end and the replicase reading the genome from its 3`- to 5`-end. Imagine how a single RNA molecule regulates all these processes sometime simultaneously and sometime at different times, sometime at a one place and sometime at different places; how all these and other functions and processes are regulated and controlled in time and space. The mystique appears to become more mysterious. Since the positive-sense RNA genomes are templates for both translation and replication, it results in interactions between host translation factors and viral RNA replication at several levels. Plus-sense RNA of plant viruses thus acts as a substrate for transcriptiton, RNA replication, translation, and encapsidation.

II. PUTATIVE 'LIFE/REPLICATION CYCLE' OF PLANT VIRUSES

Being parasites, viruses must enter and infect the host cells and exploit the cellular macromolecular machinery and sources of energy supply for their reproduction. Viral RNA replication involves both virus-encoded and cell-encoded proteins and is a very complex process. The ability of a virus to multiply requires successful virus-host interactions and certain definite host factors; involves a series of distinct and obligatory

stages that may overlap chronologically; and requires a 3`-proximal core replication promoter or simply promoter (that promotes or initiates replication), and increasingly diverse inventory of other supporting elements like replication enhancers or simply enhancer (that enhances or increases replication), replication repressors or simply repressor (that represses replication), and 5`-terminal sequences. A putative 'life cycle' of plant viruses can be built and regarded to consist of seven steps/stages, which at one time or the other overlap each other. Infection process is the **first step**. It consists of viral attachment and ingress of viral genome into the cytoplasm of host cell. This involves delivery of the virion to some extra- or intra-cellular location of the cell or initial interaction and attachment of the virus particle with a host cell and targeting of virus particles to reproductive site. The infecting virus molecule is partially uncoated at the 5`-end during the process of virus entry into a cell. Decapsidation (uncoating of viral RNA) is the **second step**. It leads to unmasking of genomic RNA by removal of coat protein by co-translational and co-replicational disassembly mechanisms thereby releasing viral genome within the cell and making it available for other functions. The genomic RNA is recruited by ribosomes and translated to produce components of viral replicase. The process of uncoating implies that second, third (synthesis of viral replicase), fourth (RNA replication), and fifth (translation of viral RNA) steps partly overlap.

The **third** step is the formation of virus-specific RNA replication proteins, including RdRp, by translation of the viral genome by host ribosomal system. It is an early event of viral replication cycle and occurs nearly at the beginning of infection. Since no pre-existing cellular machinery for replication of RNA viral genomes (that is, no RdRp) exists in host cells, therefore, all RNA viruses encode an RNA polymerase and possess a relevant gene for it. This enzyme synthesizes new RNA or DNA as well as subgenomic RNA, acts as the transcribing enzyme for transcription of RNA from DNA or RNA and has also to recruit genomic RNA (out of the translation mode) for 3`- to 5`-copying to form negative-sense RNA. This happens at RNA replication complexes associated with intracellular membranes. The replicase complex binds with the 3`-untranslated region (UTR) of genomic RNA, and the RdRp component of replicase complex employs the full-length genomic RNA as a template for synthesis of a complementary full-length minus-strand viral RNA. In fact, the study of RNA replication was hampered for a long time mainly because the active viral RdRp could not be dissociated from the host membrane and the inducible endogenous host-encoded RdRp, which is found in plant systems, could not be distinguished from the viral RNA polymerase. It was only about fifteen years back that it became possible to isolate viral RNA polymerase from infected plant tissue. This viral polymerase, in most cases, can only synthesize the RNA of negative polarity and depends on the addition of an exogenous viral template for RNA synthesis. Such RNA polymerases had been obtained for 5 viruses by 1992-1993 (David *et al.*, 1992; Ishihama and Barbier, 1994). This made it possible to investigate as to which viral proteins and host proteins participate in viral RNA replication. The availability of *in vitro* active RdRp, expressed in/obtained from heterologous systems like *Escherichia coli* (Hong and Hunt, 1996; Anindya *et al.*, 2005) and *Saccharomyces cerevisiae* (Janda and Ahlquist, 1993; Quadt *et al.*, 1995), greatly facilitated research into the mechanisms of replication of positive-sense RNA

viruses. Mutant RNA polymerases could be expressed in these systems even when they did not have to support viral RNA replication. These heterologous systems have contributed enormously to the study of plant virus replication.

The **fourth** step is the replication of viral RNA. It has several sub-steps. Firstly, initiation of RNA replication at the right template and at the right position requires specific recognition of *cis*-acting RNA elements (called replication promoters) by viral RdRp and/or associated host factors in plus-strand genomic RNA. For replication to begin, the genomic viral RNA switches (the first switch) from translation mode (during RdRp synthesis) to replication mode, indicating that viral RNAs must contain the necessary information to switch between translation and replication (Gamarink and Andino, 1998). This switch is necessary because during translation of viral genomic RNA, 5`- to 3`-ribosome trafficking blocks 3`- to 5`-polymerase copying of viral RNA. BMV genomic RNAs are recruited from translation to replication mode by a viral protein (1a, a helicase-like replication factor), which acts through *cis*-acting recognition elements of each of the viral genomic RNA (Chen *et al.*, 2001). In addition, internal locations can also bind RdRp, with RNA bridges directing the RdRp to 3`-end.

Secondly, replication of viral RNA yields progeny plus-sense RNA molecules. Viral replication involves reiterative transcription of the infecting virus plus-sense RNA template into the complementary negative-sense chain by replicase system, leading to formation of a double-stranded RNA molecule (the replicative form or RF RNA). The RF RNA is unwound by viral helicase activity, releasing the template and the single-stranded minus-sense RNA, and reiterative formation of progeny positive-sense RNA molecules by using the complementary negative-strands as templates and so leading again to the formation of a double-stranded RNA structure called replicative intermediate (RI) RNA.

Since RdRp must be recruited to 3`-ends of plus strands to initiate synthesis of full-length complementary RNAs, *cis*-acting elements thought to recruit RdRp are usually located within the 3`-noncoding regions (Duggal *et al.*, 1994). An interaction between viral polymerase and certain viral sequences that promote the minus-strand RNA synthesis begins the replication of positive-strand RNA viruses. The elements promoting negative-strand RNA synthesis are usually contained within the 3`-terminal 200 bases of many viral RNAs [as in *Brome mosaic bromovirus* (BMV), *Cucumber mosaic cucumovirus* (CMV), *Cowpea mosaic comovirus* (CPMV), *Cymbidium ringspot tombusvirus*, *Tobacco mosaic tobamovirus* (TMV) and in many other plant viruses], and include diverse forms such as tRNA-like structures, poly(A) tails, pseudoknots, small hairpins, and short primary sequences without high-order structures that have been identified as core promoters for minus-strand synthesis (Dreher, 1999). Thus, many sequences and/or structures required for negative-strand synthesis have been characterized for many RNA plant viruses. Transcription initiation site of TMV is located near extreme 3`-termini of RNA templates so that the replication promoter on positive-strand TMV RNA is located within 249-base sequence and the extreme 3`-terminal sequence of -CCCGGUAGGGGCCCA(3`) (Watanabe *et al.*, 1999). Involvement of 5`-proximal sequences and structural elements in minus-strand synthesis has also been found for an increasing number of viruses (Herold and Andino, 2001; Wu *et al.*, 2001; Vlot and Bol, 2003).

Thirdly, if RNA synthesis is initiated at internal promoters on minus-strand RNA, it leads to the formation of subgenomic mRNAs. Thus, minus strands can also act as templates for the synthesis of 3`-coterminal subgenomic RNAs by using internal promoters.

Fourthly, the synthesis of progeny positive-strand RNA occurs through asymmetric mechanism, which enables simultaneous synthesis of multiple progeny viral RNA strands that are at different stages of completion and appear to hang as tails. As many as five partially completed plus strands may be associated with RI; moreover, each full-length minus-strand RNA is used multiple times as a template for genomic or subgenomic RNA. This process results in formation of much greater number of progeny positive RNA strands so much so that the ratio of plus to minus strands at the exponential stage of replication cycle can range from 10:1 to as high as 1000:1 (Buck, 1996). This asymmetric accumulation of the minus- and plus-sense RNAs suggests the presence of a second switch, which represses minus-strand synthesis. The switch from minus-strand to plus-strand synthesis is in all probability mediated by *cis*-acting sequences that may or may not permit RdRp access to plus-strand promoter or 3`-terminal sequences. These *cis*-acting sequences and structural elements in untranslated regions of genomes allow viral RdRps to correctly initiate and transcribe asymmetric levels of plus and minus strands during replication of plus-sense RNA viruses. Such elements include promoters, enhancers, and transcriptional repressors that may require interactions with distal RNA sequences for function. The new genomic and sub-genomic RNAs are released from minus-sense template by helicase activity. Later, the methyltransferase activity of the replicase complex caps new molecules of the genomic RNA and the subgenomic mRNAs. Progeny positive RNA strands generated as above may further enter into any one the three routes of virus replication cycle: act as mRNA and code for coat protein synthesis (the translation step), be converted to double-stranded RF RNA to provide more templates for greater progeny positive RNA synthesis, or be encapsidated to produce the virions (the encapsidation step). Pulse chase experiments and kinetics of labeling were the tools of choice for these studies.

For asymmetric viral RNA replication, viral RNAs must have information to support asymmetric synthesis of plus and minus strands. Requirements for this include transcriptional enhancers (Nagy *et al.*, 1999; Pogany *et al.*, 2003; Ray and White, 2003) and repressors that function via RNA-RNA (Pogany *et al.*, 2003) or protein-RNA (Dreher, 1999; Zhang *et al.*, 2004a, 2004b) interactions.

The promoter regions necessary for minus-strand RNA and subgenomic RNA synthesis have been mapped in several positive-strand RNA viruses. These two types of promoters generally do not share common structural characters despite their being recognised by the same viral polymerase. In fact, the single type of polymerase produced by a particular virus possesses the ability to recognise different types of promoters of that virus even though little similarity occurs in sequence and structure between these promoters located on a viral genome (Miller and Koev, 2000). The subgenomic RNA promoter is generally situated 3`-with respect to the transcription start site while the plus- and minus-strand genomic RNA promoters, so as to prevent loss of genetic information, are located 5`-of their start site. Enhancers and repressors regulate promoter efficiency and may be located either proximal or distal to core

promoter (Nagy *et al.*, 1999, 2001; Panavas and Nagy, 2003; Pogany *et al.*, 2003; Ray and White, 1999, 2003).

The progeny positive RNA strands generated as above may further enter into any of the three routes of virus replication cycle: act as mRNA and code for viral proteins (the translation step), be converted to double-stranded RF RNA to provide more templates for greater progeny positive RNA synthesis, or be encapsidated to produce the virus particles (the encapsidation step).

The **fifth** step is the translation of viral genomic RNA when it directly serves as mRNA and produces structural and non-structural proteins by exploiting cellular functions. Virus-specific capsid is the structural protein while various enzymes are the non-structural proteins. To compensate for the limited amount of genetic information and to maximize their coding capacity, plant viruses have developed a great number of highly sophisticated strategies to synthesize their proteins. These strategies are: synthesis of subgenomic RNAs, use of polycistronic RNAs or of ambisense RNAs, RNA splicing, internal in-phase initiation, gene overlap, readthrough of termination codons, shift in reading frame and post-translational cleavage of a polyprotein. Translational programming permits the viruses to conserve the functional genome size by making efficient use of genome coding capacity.

Viral assembly/encapsidation of progeny plus-sense RNA molecules by capsid protein subunits to produce progeny virus particles is the **sixth** step. It involves recognition of the specific progeny genomic RNA by the specific capsid protein subunits and the assembly of viral RNA with its specific proteins to form complete virus particles. Empty capsids are also formed in some cases. Maturation of virus particles is the **seventh step**. However, it does not occur in plus-sense RNA viruses but occurs in enveloped viruses, which acquire the envelope by budding through some membranous system. Progeny virus particles, subsequent to their formation, spread within the host followed by release outside the host and/or transmission of virions from infected cells to next host in which the infection cycle is repeated. Successful infection involves formation of 'virus replication factories' on cell membranes and transport of such factories within and between cells.

Each of the above steps can occur at one or more specific sites and is a complex phenomenon requiring specific conditions. Some of these stages overlap chronologically. No natural DNA phase is involved in replication of positive-sense RNA plant viruses, this replication process shares fundamental similarities with replication phenomenon of cellular RNA, and the positive-strand RNA viruses possess a common replication strategy (Pogue *et al.*, 1994).

Synthesis of cellular RNA is DNA-dependent, and hence is called DNA-dependent RNA synthesis. However, most of the eukaryotic cells do not contain pre-existing machinery for conducting RNA-dependent RNA synthesis and so are unable to replicate RNA viral genome. To overcome this inability, all RNA viruses encode an RNA polymerase within the invaded cells so as to make the viruses competent for multiplication of their RNA genome. This enables the viruses to replicate and transcribe their genomes in eukaryotic cells by frequently subverting cellular factors.

These subverted factors play an integral and regulatory role in replication and transcription of viral RNA.

Viral RNA synthesis has been conducted both *in vitro* and *in vivo.* Generally, an *in vitro* system of RNA synthesis can be used to dissect the mechanism and roles of proteins involved in RNA replication. However an *in vitro* system may lack important properties that can only be found *in vivo,* since some host proteins are involved in the replication process (Lai, 1998; Ahlquist *et al*., 2003). While *in vivo* results could be predicted from the *in vitro* results*,* the reverse is not always true. Some differences exist in the influence of stem-loops on replication of *Hibiscus chlorotic ringspot virus* between the *in vitro* and *in vivo* systems (Wang and Wong, 2004). Deletion of U loop of stem-loop 2 slightly affected the replication efficiency *in vitro* but the mutant could not replicate in knead protoplasts. The requirements of stem-loops for minus-strand RNA synthesis of TMV carried out *in vitro* were the same as in tobacco protoplasts (Osman *et al.*, 2000). However, differences were noted in replication efficiency of some TMV mutants *in vivo* (Chandrika *et al.*, 2000). Similarly, insertion of 3 nucleotides in stem-loop C of BMV RNA3 showed reduced RNA synthesis *in vitro* but not *in vivo* (Sivakumaran *et al.*, 2003). Therefore, *cis* sequence elements and structural requirements for RNA synthesis need to be analysed both *in vitro* and *in vivo.*

Replication of viral RNA begins with specific recognition of *cis*-acting RNA elements on the infecting positive-sense RNA by viral RdRp and/or associated host factors leading to synthesis of complementary negative-sense intermediates, that later act as templates for synthesis of plus-sense RNA progeny molecules. Therefore, RNA synthesis is regulated by sequences present on both positive- and negative-stranded RNA templates and the essential *cis*-acting motifs have to include the promoters for synthesis of the negative strand from the infecting positive-strand RNA and synthesis of the positive-strand progeny RNA molecules from the negative-strand templates.

A. Core Promoters

Core promoters, present in viral RNAs, contain multiple sequence and structural features that are essential for efficient recognition of viral RNA by cognate RdRp (Kim *et al.*, 2000). They recruit viral RdRp to transcription site, are located at the 3`-ends of positive-strand RNA (and also negative-strand RNAs), are generally constituted by one or a few 3`-proximal hairpins that specifically interact with polymerase for initiation of minus-strand RNA synthesis, are generally located proximal to 3`-terminal sequences, and are usually comprised of one or more hairpins with adjoining single-stranded sequence (Duggal *et al.*, 1994; Song and Simon, 1995; Buck, 1996; Chapman and Kao, 1999; Dreher, 1999; Sivakumaran *et al.*, 1999). However, some viral RNAs are able to replicate in the absence of large 3`- or 5`-terminal sequences (Wu and White, 1998), which suggests that promoter sequences may not be required for them. All core promoters permit basal levels of RNA transcription; however, efficient RNA synthesis requires additional viral elements like structures and sequences at 5`-ends that may be needed for genome circularization (Herold and Andino, 2001; Khromykh *et al.*, 2001) and internal elements such as enhancers, promoters, repressors, and RNA chaperons,

which function either in *cis* (Nagy *et al.*, 1999, 2001; Ray and White, 1999, 2003; Vlot *et al.*, 2001; Panavas and Nagy, 2003; Pogany *et al.*, 2003; Zhang *et al.*, 2004a, 2004b) or in *trans* (Sit *et al.*, 1998; Eckerle and Ball, 2002). Genomic RNAs of all carmoviruses, except of *Galinsoga mosaic virus*, contain a 3`-proximal hairpin that comprises the core promoter for minus-strand RNA synthesis (Song and Simon, 1995). The best-characterized BMV core promoter is the one that directs initiation of minus-strand RNA synthesis.

B. Replication Complexes

It is being increasingly realized now that it is not simply the replicase molecules that are involved in viral RNA replication but rather a complex entity called replication complex. Replication complexes are known/regarded to be constituted by several of the following elements: virus-encoded proteins [the RdRp itself that is characterized by a conserved Gly-Asp-Asp (GDD) motif and the auxiliary proteins], an nucleoside 5`-triphosphate (NTP)-binding RNA helicase motif in the same or in another protein in nearly all cases, methyltransferase, host-derived proteins/factors and the RNA template (Buck, 1996, 1999; Osman and Buck, 1997; Lai, 1998; Kao *et al.*, 2001; Ahlquist *et al.*, 2003). In fact, replication complexes of some, if not of many positive-strand RNA plant viruses, are considered to sequester viral RNA templates along with replicases, putative RNA helicases and capping factors (Schwartz *et al*., 2002). Thus, replication and transcription in positive-strand RNA viruses use a complex of viral and cellular proteins - the replication complex - that initiates RNA synthesis at 3`-end of genomic RNA. Such replication complexes have been detected in plants infected with BMV, CMV, *Cucumber necrosis virus* (CNV), TMV, and many other plant viruses. Specific and complex interactions between viral genomic RNAs and viral replicase are required for replication and/or transcription of viral RNAs. Viral replication complexes are treated in detail in a later chapter.

C. Switches

The changeover of one stage of RNA replication to the next is a controlled event, although we do not yet understand this controlled switchover. The first switch is regarded to be operative subsequent to formation of RdRp by ribosomes; it ensures that the genomic viral RNA switches from translation mode (during RdRp synthesis) to replication mode. For this, RdRp and possibly other viral or host factors form an initiation complex at the promoter and initiate complementary minus strand. The second switch is considered to be the one that ensures asymmetric accumulation of minus- and plus-RNA strands; it represses minus-strand synthesis, thereby constraining the RdRp to synthesize minus strands but favours synthesis of plus-strands of RNA. This switch from minus-strand to plus-strand synthesis is likely mediated by *cis*-acting elements that allow or deny access of RdRp to the plus-strand promoter or 3`-terminal sequences (Sun *et al.*, 2005).

Regulation of switches is carried out by some plant viruses by changing the conformation of 3`-proximal structures and this may be mediated by one or more

unstable base pairs occurring between complementary short sequences located within and outside hairpins (Olsthoorn *et al.*, 1999; Koev *et al.*, 2002; Pogany *et al.*, 2003; Zhang *et al.*, 2004a; Sun *et al.*, 2005). For example, repression of minus-strand RNA synthesis by *Barley yellow dwarf virus* occurs by altering the conformation of its 3`-end to a 'pocket' structure in which the transcription initiation site is unavailable to RdRp since the site is embedded in a stem (Koev *et al.*, 2002). Moreover, apart from the *cis*-acting sequences, *trans*-acting cellular factors or virus-encoded proteins could also influence the balance between alternative structural conformations (Olsthoorn *et al.*, 1999). Thus, the presence of alternative structural conformation of some viral 3`-ends may be one mechanism to limit minus-strand synthesis and permit greater synthesis of progeny plus strands.

Another type of switch controlling virus replication has been found during studies on replication of BMV RNA. It is the mutual interference by and influence of different types of viral RNAs on each other during their synthesis. BMV RNA3 acts as a template for RNA replication as well as for sgRNA transcription. Grdzelishvili *et al.* (2005) studied replication of BMV RNA3 and formation of subgenomic RNA (sgRNA) in *Saccharomyces cerevisiae* expressing 1a and 2a replication proteins and which support full RNA3 replication cycle. They observed two main and opposing trends: firstly that blocking of sgRNA transcription (that is, inhibiting sgRNA synthesis) stimulated RNA3 replication by up to 350%, which shows that sgRNA transcription inhibits RNA3 replication. This inhibition only operated in *cis* and was independent of the product of sgRNA synthesis that is the capsid protein. Secondly, transcription/formation of sgRNA inhibited RNA3 replication at a step or steps after negative-strand RNA3 synthesis, implying competition with positive-strand RNA3 synthesis for negative-strand RNA3 templates, viral replication factors, or common host components. Accordingly, inhibition of positive-strand RNA3 synthesis stimulated sgRNA transcription by up to 400%. They concluded that BMV sub-genomic transcription and genomic RNA3 replication/synthesis mutually and strongly interfere with each other *in vivo.* It is the sgRNA synthesis itself and not sgRNA promoter sequences or products of sgRNA synthesis that inhibit RNA3 replication. Strikingly, this inhibition of sgRNA synthesis specifically targeted replication of its parental RNA3 but not separate RNA3 derivatives replicating in the same cell.

Hema and Kao (2004) and Sivakumaran *et al.* (2004) studied interaction of sgRNA and genomic RNA3 synthesis in barley protoplasts transfected with BMV RNAs 1 to 3. These studies also indicated interference of positive-strand RNA3 synthesis with sgRNA transcription (Hema and Kao, 2004) but there was one difference from the results of Grdzelishvili *et al.* (2005): inhibition of sgRNA transcription did not stimulate RNA3 accumulation in transfected protoplasts; rather, in many cases, inhibiting sgRNA transcription reduced RNA3 accumulation relative to that of wild type due largely to blocking capsid protein (CP) expression. Grdzelishvili *et al.* (2005), in line with the results of Hema and Kao (2004) and Sivakumaran *et al.* (2004), found that blocking sgRNA synthesis often has less effect on RNA3 accumulation in the barley protoplast system than in yeast.

Grdzelishvili *et al.* (2005) suggested that the mutual interference between sgRNA transcription and positive-strand RNA3 synthesis could be due to their competition for negative-strand RNA3 template, viral replication factors and for one or more common,

limiting components of the BMV RNA synthesis machinery, or limiting host components. This competition is understandable since synthesis of both sgRNA and positive-strand RNA3 occurs in 50- to 70-nm-diameter endoplasmic reticulum (ER) membrane-bound RNA replication compartments, use the same negative-strand RNA3 template, requirement for BMV replication factors 1a and 2a, and have similar replication kinetics (Ishikawa *et al.*, 1997).

III. *CIS*-ACTING AND *TRANS*-ACTING VIRAL NUCLEOTIDE SEQUENCES

Positive-strand RNA viruses contain *cis*- and *trans*-acting nucleotide sequences, which affect and perform indispensable roles in viral RNA replication, in their 5`- and 3`-UTRs (Duggal *et al.*, 1994; Buck, 1996; Lai, 1998; Héricourt *et al.*, 1999). These nucleotide sequences have been referred to in literature by several interchangeable terms: elements/factors/motifs/sequences/signals/structures. In *cis* reaction, the nucleotide sequences and their site of action are contained in the same molecule; hence such a reaction is monomolecular. In *trans* reaction, the nucleotide sequences and their site of action are contained in separate molecules; hence this reaction is bimolecular.

A. *cis*-Acting Sequences

The site of action of the *cis*-acting motif may be near to or distal from the site where this motif is located. The *cis* reactions are generally very fast, occur cotranslationally, are also called 'autocatalytic' or 'autoproteolytic', and are functional in all stages of virus replication so that different stages of viral RNA replication have their own specific *cis*-acting sequences. The best-known *cis*-acting elements are the positive-strand viral RNA replication and transcription promoters, which are essential for initiation of RNA synthesis and formation of negative-strand RNA synthesis by specific RdRps. Many such *cis* sequences have been characterized in several RNA plant viruses (de Graaff and Jaspars, 1994; Buck, 1996; Lai, 1998; Héricourt *et al.*, 1999; Mandahar, 1999). The *cis* factors needed for formation of plus-strand progeny RNA and replication complexes have also been distinguished/predicted.

The *cis*-acting signals needed for RNA replication are always located on viral RNA genomes and are generally found at both the 3`- and 5`-UTRs of the RNA genomes. The 3`-terminal *cis*-acting elements are essentially required for faithful recognition and replication of only the cognate RNA by specific viral polymerases; have been characterized in many plant viruses; and contain poly(A) tails, pseudoknots, tRNA-like structures, stem-loop structures, hairpins or short primary sequences without high order structures. However, the *cis*-acting replication elements also exist in protein-coding regions as in *Tomato bushy stunt virus* (TBSV) (Ray and White, 2003). Several internally placed *cis*-acting elements are also known as in BMV and in other plant viruses (Duggal *et al*., 1994; Buck, 1996; Dreher, 1999). More complicated cases of *cis* factors also exist where internal elements have a long-distance interaction

with terminal sequences for efficient positive-strand synthesis as in *Potato virus X* (PVX) genomic RNA (Kim and Hemenway, 1999). Moreover, some *cis*-acting elements function as RNA chaperons and assist in replicase maturation (Quadt *et al.*, 1995; Vlot *et al.*, 2001). Thus, a large variety of *cis*-acting elements occur ranging from short linear nucleotide sequences to extensive multiple stem-loop structures. Plant viruses may contain several of these sequences. For example, BMV possesses three different types of promoters for different functions. One is the tRNA-like structure at 3`-end of genomic RNAs and contains motifs required for negative-strand synthesis. Second promoter is located at the 3`-terminus of negative-strand intermediates and is required for synthesis of genomic positive-strand RNA molecules. Third is the subgenomic RNA promoter, which is located internally on the genomic RNA. Similar replication enhancers also exist in several other plant viruses like *Alfalfa mosaic virus* (AMV), *Turnip crinkle virus* (TCV), TBSV, etc. and must be existing, but not yet identified, in many other plant viruses. Apart from the above well-defined and definite *cis*-acting structures, other *cis*-acting elements (involved in RNA replication) occur in many plant viruses and do not seem to possess easily defined sequence motifs.

It seems that RNA replication promoters (in TCV) are organized in a 'modular fashion', contain an RNA replication enhancer sequence and a replication initiation sequence. The segment A (a sub-element of region III of TBSV RNA) also behaves as a modular element (Ray and White, 2003). Both the activator proteins (of transcription and splicing) possess a common modular organization that has separate nucleic acid binding regions as well as regions that bind proteins by specific protein-protein interactions.

1. Internal Control Regions (Internal *cis*-Acting Sequences)

The internal control regions (ICR)-like motifs, frequently found in RNA genomes of plant viruses, act as promoters of viral positive-strand RNA synthesis. Marsh and Hall (1987) and French and Ahlquist (1987) discovered that 5`-untranslated regions of each BMV RNA and the intercistronic region of BMV RNA3 contain sequences that closely resemble the internal control regions (ICRs) 1 and 2 of tRNA genes. These sequences have been called by various names in literature - internal sequences/intergenic motifs or sequences/intercistronic regions/internal *cis*-acting sequences/internal control regions or motifs, The ICR-like sequences are present among BMV RNA 1; CMV RNAs 1, 2, and 3; TMV RNA; *Turnip yellow mosaic tymovirus* (TYMV) RNA and other plant virus genomes. These motifs are always placed within the known or putative promoter sequences involved in viral RNA replication.

The intercistronic region of BMV RNA3 occurs between genes for 3a protein and capsid protein, and contains one ICR-1 motif, three ICR-2 motifs and the intercistronic *cis* elements. The ICR-2-like motif starts at base 1100 in the intercistronic region and is almost identical to those present at the 5`-termini of BMV RNAs 1 and 2 and the tRNA-like consensus. Also, the 3`-most ICR-2 motif of the intercistronic region bears core promoters for subgenomic RNA formation. Pogue and Hall (1992) proposed that

the ICR motifs at the 5`-end of RNA2 are part of a stem-loop structure such that the ICR2 domain is present in the loop region while the ICR1 domain is part of the stem.

Multiple consecutive C residues, contained in all the above sequences (Song and Simon, 1995; Wang and Simon, 1997), are arranged in a linear fashion and also occur in a hairpin replication enhancer motif located on negative strands of TCV satellite RNA C. Perhaps, this 5`-proximal linear replication enhancer element attracts RdRp to template (the negative-strand of TCV satellites RNA C) (Nagy *et al*., 1998, 1999) and is required for synthesis of positive-strand of the satellite RNA C (Guan *et al*., 2000). The above linear RNA element resembles BMV subgenomic RNA core promoter in which four (at positions -17, -14, -13, and -11) of the 22 nucleotides are essential for recognition by BMV RdRp *in vitro* (Siegel *et al*., 1997, 1998). Two motifs (ICR1 and ICR2), located at 5`-terminal region of genomic RNAs of *Bromoviridae*, bind to cell transcription factor that has significant role in formation of initiation complex for synthesizing positive-strand RNA.

A 351-nucleotide segment, located upstream of an AUG that is distal to the first initiation codon in cowpea mosaic virus M component RNA, permits internal initiation at the distal AUG. Similarly, the 5`-leader of turnip mosaic potyvirus RNA or the nucleotide sequence located upstream of the 3`-end capsid protein subgenomic mRNA of a crucifer-infecting tobamovirus also permit internal initiation (Basso *et al*., 1994; Ivanov *et al*., 1999).

A *cis*-acting replication motif is located internally within the 3`-UTR of tombusviruses. It is called region III (Ray and White, 2003), has been characterized, and is postulated to have enhancer-like function during tombusvirus replication. The negative-stranded region III functions as a 'strong' replication enhancer and it increases RNA synthesis by 10-to-20-fold as compared to other viral sequences (Panavas and Nagy, 2003). These workers concluded that the replicase enhancer located in region III functions as a strong enhancer in negative-stranded RNA and a weak enhancer in positive-stranded RNA of tombusviruses. Ray and White (2003) also arrived at the same results concerning the role of region III in enhancing tombusvirus replication. Panavas and Nagy (2003) establish that region III of tombusviruses contains a replication enhancer that directly facilitates *de novo* RNA synthesis from minimal promoters; that negative-strand region III is a stronger replication enhancer than positive-strand region III; and that their *in vitro* studies support the results of *in vivo* studies of Ray and White (2003). Intergenic regions of RNA genomes of *Barley stripe mosaic virus* (Zhou and Jackson, 1996), and *Cucumber mosaic virus* (Boccard and Baulcombe, 1993) contain *cis* elements.

2. Role of *cis* Factors

Three functions have been attributed to the *cis*-acting elements: to recognize and then replicate cognate RNA, to recruit viral RdRp, and to facilitate *de novo* initiation of complimentary strand RNA synthesis. In addition, the RNA *cis*-acting sequences may also perform important roles during RNA recombination, viral evolution and virus adaptation. The role of the internal *cis*-acting motifs has not been well established but it could be RNA folding, binding of host factor, and assembly of the replicase complex

(Buck, 1996; Kim and Hemenway, 1999). Mostly, the *cis*-acting elements seem to function through direct interaction with other sequence elements or viral or cellular proteins (Duggal *et al.*, 1994; Dreher, 1999; Sivakumaran *et al.*, 1999; Fabian *et al.*, 2003).

The distal *cis* sequences and/or structural elements affect ribosomal frameshifting, transcription, and translation in *Barley yellow dwarf virus* (Koev *et al.*, 1999; Guo *et al*., 2000; Paul *et al.*, 2001). Stem-loop structures have great importance for transcription of TMV subgenomic RNA (Grdzelishvili *et al*., 2000).

Mechanisms for transcription and splicing regulation require special *cis*-acting RNA sequences that promote and regulate activities of transcription or splice sites during transcription and RNA splicing, respectively. The promoters get associated with regulatory proteins to constitute multicomponent promoter/enhancer complexes that recruit the relevant enzymatic machinery to sequences that recognize the promoter or splice sites. During recruitment, direct interactions occur between the regulatory proteins in the enhancer complexes and the components of the basic enzymatic machineries. The high degree of regulatory specificity during transcription and splicing is largely due to the multicomponent nature of the enhancer complexes and to their cooperative assembly. Specific activator proteins recognize transcription enhancers while members of serine-arginine-rich protein family mostly recognise the splice enhancers.

Exact mechanisms of functioning of the enhancers during transcription and splicing phenomena are not well understood. However, the two processes have certain common features: cooperative binding of regulatory proteins to promoters forms highly stable complexes that recruit the basic components of transcription or splicing machinery to the nearby recognition motifs; regulation of transcription and splicing occurs by similar ways which include multicomponent nature of enhancer complexes, and modular organization of the enhancer-binding proteins.

The ICR-like motifs found in RNA genomes of plant viruses possibly act as promoters of viral positive-strand synthesis.

A role of RNA polymerase III involved in tRNA synthesis can be suggested in viral RNA replication.

Thus, some of the different steps in viral replication regulated by specific *cis*-acting elements (present in both plus- and negative-strand RNA templates) are: assembly of a replicase activity on inoculum RNAs, formation and recognition of replication complexes, transcription of the incoming infecting viral RNA genome, formation of positive-sense RNA from the negative-sense transcripts, increased translation of RNA, splicing of the translated polyproteins, cap-independent translation, assembly of virus particles, recognition of site for replication proteins, synthesis of genomic and subgenomic RNAs; and in even still other stages.

3. *cis*-Acting Sequences in Brome mosaic bromovirus RNAs

The *cis*-acting elements for efficient BMV genomic plus-strand, minus-strand, and subgenomic RNA synthesis have been characterized and a number of required *cis* elements have been identified. Choi *et al.* (2004) have given a summary of the known

cis-acting sequences in BMV RNAs and the following information is based on this paper.

Plus-sense RNA1 contains the regulatory element called B-box located at +17 to +30 nucleotides, and stem-loop C (SLC) (within tRNA-like structure) located at +3156 to +3186 nucleotides. The 3`-noncoding regions of BMV genomic plus-strand RNAs form a tRNA-like structure that directs the initiation of minus-strand RNA synthesis *in vitro* and *in vivo*. The SLC of RNA3 binds BMV replicase through an RNA structure known as clamped adenine motif. Highly similar structures are predicted for RNA1 and RNA2, so that most likely the same structures are required to direct their minus-strand replication.

Negative-sense RNA1 contains complementary B-box (cB-box) located at –3204 to –3217 nucleotides. The core of cB box is a 4-nucleotide sequence, CCAA, which is conserved at comparable positions in RNA1 and RNA2 of other members of *Bromoviridae.* The cB box is the sequence complementary to the previously identified box B regulatory element.

Plus RNA2 contains B-box located at +17 to +30 nucleotides, and SLC located at +2787 to +2817 nucleotides.

Negative RNA2 bears cB-box located at –2835 to –2848 nucleotide.

Plus RNA3 bears a B-box-like structure located at +23 to +36 nucleotides, replication enhancer located at +1012 to +1221 nucleotides, replicase assembly site located at +1003 to +1217 nucleotides, B-box located at +1100 to +1113 nucleotides, and SLC located at +2039 to +2069 nucleotides.

Negative RNA3 contains minimal promoter located at –2092 to –2117 nucleotides, and subgenomic core promoter located at –876 to –895 nucleotides.

B. *trans*-Acting Viral RNA Nucleotide Sequences

Four satellite viruses exist in association with their helper viruses in natural plant infections: *Satellite maize white line mosaic virus* (SMWMV), *Satellite panicum mosaic virus* (SPMV), *Satellite tobacco mosaic virus* (STMV), and *Satellite tobacco necrosis virus* (STNV) (Dodds, 1998; Scholthof *et al*., 1999). Satellite RNAs are dependent upon the respective helper viruses for replication, encapsidation and dissemination (Taliansky and Palukaitis, 1999). Helper viruses recognise the respective satellite viruses/RNAs in *trans* so that the helper viruses and the host plants have to provide all *trans*-acting factors necessary for replication of satellites (Qiu and Schlothof, 2000; Song and Miller, 2004).

This recognition of satRNAs in *trans* by their respective helper virus replicase complex is ensured mainly by sequences/signals present in their 5`- and 3`-UTRs (Routh *et al*., 1997; Bringloe *et al*., 1998). The *cis*-acting elements essential for STNV replication are embedded in three hairpin-like structures on 5`-UTR, two discrete regions identified as 3`-proximal terminal sequences, and the sequences immediately adjacent to capsid protein open reading frame (ORF) in 3`-UTR (Bringloe *et al*., 1998). Similarly, the 3`-UTR sequence immediately downstream of capsid protein ORF is critical for accumulation of STMV (Routh *et al*., 1995, 1997). On the other hand,

capsid protein genes of both STNV and STMV are not essential for their accumulation and movement (Bringloe *et al*., 1998; Routh *et al*., 1995, 1997). Signals for replication and movement of SPMV RNA are located in 5`-proximal nucleotides - namely nucleotides numbers 62 to 110 in 5`-end, the entire 3`-UTR, and 73 nucleotides upstream of capsid protein stop codon.

The RNA1 of bipartite *Lettuce infectious yellows virus* (LIYV) encodes a *trans* enhancer for RNA2 accumulation (Yeh *et al.*, 2000). The RNA1 3`-terminal ORF2 encodes a protein p32 that acts as the *trans* enhancer of LIYV RNA2 accumulation. This LIYV RNA1-mediated *trans* replication enhancer activity (through p32) affecting accumulation of LIYV RNA2 is unusual among RNA plant viruses.

Red clover necrotic mosaic virus (RCNMV) is a bipartite virus and its genome consists of RNA1 and RNA2. Capsid protein is translated from a subgenomic RNA transcribed from RNA1. Sit *et al*. (1998) showed that a 34-nucleotide sequence in RNA2 is required for transcription of subgenomic RNA (from RNA1 of RCNMV) and they proposed a model in which RNA2 directly binds to RNA1 and *trans*-activates synthesis of subgenomic RNA. This regulation of transcription by RNA mediation is not a usual process in RNA viruses that normally rely on protein regulators. The *trans*-acting RNA element required for subgenomic RNA synthesis from RNA1 is located in protein-coding region of RNA2; however, RNA2 is not required for repliation of RNA1. It is the stem-loop structure SL2 of 20 nucleotides (of RNA2) that functions as a *trans*-activator for subgenomic RNA synthesis (from RNA1) (Sit *et al.*, 1998; Guenther *et al.*, 2004; Tatsuta *et al.*, 2005). Thus, SL2 is responsible for RNA-mediated *trans*-activation of subgenomic RNA transcription from RNA1 through direct pairing between RNA1 and RNA2. Simultaneously, SL2 is also a *cis*-acting RNA2 replication element; in fact, SL2 of RCNMV RNA2 has at least three different functions: as a *cis*-acting sequence for replication of RNA2, as a *trans*-acting sequence for capsid protein expression through production of subgenomic RNA, and as a coding sequence for movement protein (Tatsuta *et al.*, 2005).

IV. HOST GENE SHUT-OFF AND HIJACKING OF HOST CELLULAR MACHINERY BY VIRUSES

Viruses have developed the capacity to use machinery of the host cell for synthesis of their own DNA and RNA (during the viral nucleic acid replication process), and proteins (during the translation process) and utilize the cellular metabolic machinery to produce viral proteins and viral genome. DNA viruses have an inherent advantage here; they can use the already existing host cell machinery, which replicates and transcribes the host DNA, for replication and transcription of their DNA. In contrast, since no pre-existing cellular machinery exists in host cells for replication of RNA viral genomes, all RNA viruses encode an RNA polymerase. In fact, close integration between viral and host factors suggests the virus-infected cell to be a unified entity that constitutes the functional unit of infection (Ahlquist *et al*., 2003).

There is some evidence about the way plant viruses out-compete cellular processes of the infected cells. The 3`-untranslated region of AMV RNA4 has a role in translation initiation and is also required for efficient competition of virus RNA4 with

cellular messenger RNAs in wheat germ extracts and oocytes (Hann *et al*., 1997). Moreover, many viral mRNAs have developed strategies of translation enhancement that are different from those used by most cellular mRNAs. Typically, cellular mRNAs have a terminal cap and a poly(A) tail which interact synergistically and function as co-dependent regulators of translation by interaction between these termini of mRNAs (Gallie, 1996, 1998). This happens in many of the plant viruses also but cap is absent in a significant number of plant viruses so that they exhibit cap-independent translation mechanism. Enhanced translation of specific viral mRNAs leading to high levels of protein synthesis of specific viral genes in plants may be a fundamental mechanism by which viral mRNAs out-compete their cellular counterparts (Qu and Morris, 2000).

Viruses can down-regulate the expression of cellular genes, an effect that has been called host gene 'shut-off' by Aranda and Maule (1998). Host shut-off is the process that results in suppression of cellular macromolecular synthesis after infection by many but not all viruses, is caused by viral domination of host metabolism and so is a virus-directed process, favours selective translation of viral mRNAs over that of the endogenous mRNAs, is partial and never absolute, and is not always required for virus replication. This selective translation of viral mRNAs during host shut-off is due to a general competition between viral and host mRNAs for host translation machinery. There was a complete transient inhibition of expression of at least 10 different cellular genes in host cells associated with replication of *Pea seed-borne mosaic potyvirus* (PSbMV) (Wang and Maule, 1995). PSbMV transiently suppressed the expression of host genes in cells most active in viral replication and these cells formed a band encompassing between four to eight cell layers. Restrepo-Hartwig and Carrington (1992) had suggested, but not proven, that nuclear inclusion proteins a and b (NIa and NIb) of *Tobacco etch virus* (TEV) may affect host gene expression, ribosome assembly, or some other nuclear event. In fact, host shut-off is now considered a common phenomenon associated with animal and plant virus replication. It is not yet certain whether host shut-off is a prerequisite for virus multiplication or is an indirect consequence of viral protein function. Simultaneously, there is more than a compensatory increase in protein in cells just behind the infection front (Wang and Maule, 1995).

The host shut-off process is selective and never all-inclusive because all viruses need some components of host pathways for genome replication and expression. Thus, despite the down-regulation of host gene expression, some host genes escape shut-off and may even show coordinate induction. In PSbMV-infected peas, many host mRNAs were removed from the infected cell while HSP70 and polyubiquitin mRNA accumulation increased; these host RNAs were translated to give increased protein in the shut-off zone. These induced host genes, like the viral RNA, have the ability to escape shut-off.

Host shut-off may have some consequences for the host cell as well. However, no obvious cytopathic effect was apparent in cells showing shut-off (Wang and Maule, 1995; Aranda *et al.*, 1996) but electron microscope studies showed a rapid but transient loss of ER linked to shut-off onset (Roberts *et al.*, 1998). The long-term effect of shut-off was neither cell lysis nor necrosis. However, transient shut-off in infected cells could act as a trigger for a series of physiological changes manifested later as virus-induced

symptoms (Técsi *et al.*, 1996). Other proteins also increase during plant virus infection (Aranda *et al.*, 1996; Havelda and Maule, 2000). Increase in protein expression could prepare the cell for biosynthetic demands of viral infection.

A. Mechanisms of Shut-off

Host shut-off has been investigated in far more detail in animal than plant viruses (Aranda and Maule, 1998; Bushell and Sarnow, 2002); hence the examples given here more often pertain to the animal viruses. The precise mechanism(s) for achieving host shut-off vary widely in different virus infections and has been attributed to various factors including perturbation of intracellular ion concentration, altered nucleotide metabolism, alterations in RNA stability, changes in processing and recruitment of specific host factors, alterations in nucleocytoplasmic transport of mRNAs, modifications in cellular transcription process, and alterations in cellular translation machinery and mechanism. Host shut-off can function at different points of the eukaryotic gene expression pathway and any of the key control steps on the pathway can serve as targets for shut-off.

Poliovirus replicates in cytoplasm but causes shut-off of nuclear functions since it blocks cellular transcription by inhibiting RNA pol II transcription by its protease $3C^{pro}$. Thus, decrease in host RNA synthesis in *Poliovirus*-infected cells is correlated with degradation of transcription factors by viral serine protease 3C, which causes a cleavage of the cellular translational component p220. The mRNA maturation is another method. *Herpes simplex virus-1* (HSV-1) replicates in nucleus but influences host mRNA maturation through the action of its IE63 (also called ICP27) gene product. This results in reduced accumulation of host spliced mRNA. Thus, HSV-1 disrupts the cellular splicing machinery so as to promote preferential maturation of its own transcripts.

Modification of nucleocytoplasmic transport of mRNA is still another method by which viruses affect host shut-off. *Vesicular stomatitis virus* (VSV) (a negative-sense RNA virus) controls nucleocytoplasmic transport of host mRNAs. The virus replicates in cytoplasm but its matrix (M) protein localizes to nucleus of infected cell and effectively blocks export of host mRNAs and proteins from nucleus in *Xenopus laevis* oocytes. The M protein interferes with transport mechanisms dependent on guanosine triphosphatase (GTPase) and its associated factors. In contrast, subgroup C human adenoviruses, which replicate in nucleus, act in another way. Most cellular mRNAs, although transcribed, fail to enter cytoplasm efficiently during the late phase of infection, whereas viral mRNAs and some cellular RNA species are translocated. This selective block requires the synthesis of viral E1B-55K and E4 proteins.

Influence of viruses on stability of host mRNA(s), like decreasing the stability of mRNA by HSV-1, causes host shut-off. Such degradation of host mRNAs is widespread in animal viruses and has also been detected in a plant-infecting potyvirus, PSbMV. Many host mRNAs, presumably by degradation, are lost very rapidly following the onset of viral RNA replication (Wang and Maule, 1995). There is some selectivity in this process since some host mRNAs accumulate and are translated.

Deleterious effect of virus infection on translation process of the host cell is the most important cause of host shut-off. Translation of viral mRNAs by host machinery occurs essentially as that of cellular mRNAs (Gale *et al.*, 2000; Thompson and Sarnow, 2000) and one of the first steps is the recruitment of mRNAs by translation initiation factor eIF4F complex. But, an invading virus does not contain functional ribosomes so that for successful amplification of its genomes, a virus mRNA has to compete with cellular mRNAs for host cell translation machinery and for other functions. Several RNA viruses have developed remarkable strategies to do so. Bushell and Sarnow (2002) review the mechanisms by which viruses take over the translational machinery of the host cell. The most studied example of a block in translation involves animal picornaviruses and is reviewed by Bushell and Sarnow (2002). Recruiting the host translation initiation factors for translation initiation by the invading virus is one such step. This is done in three different ways. Cleavage of poly(A)-binding protein (PABP) and disruption of closed-loop translation complex is one such method. This happens by proteolysis/ cleavage of PABP and eukaryotic initiation factor 4G (eIF4G that is a subunit of eIF4F**)** and results in cessation of translation initiation. This process is adopted by picornaviruses. Poliovirus $2A^{pro}$ causes this disruption. Host mRNA translation through modulation of PABP, which is a 70-kDa RNA-binding protein that plays a direct role in mRNA stability through its interaction with poly(A) tails of mRNAs. The circularization of translation initiation complex is facilitated through PABP interaction with mRNA poly(A) tail and eIF4G subunit. The resulting 'closed loop' translation initiation complex is thought to stabilize assembled initiation factors and increase translation efficiency. Thus, viral disruption of PABP function can be expected to alter mRNA stability and reduce the overall rate and efficiency of cap-dependent mRNA translation. Moreover, the modified eIF4F becomes inactive for cap-dependent translation of cellular mRNAs but remains functional for translation of uncapped viral RNAs by internal ribosome entry as in picornaviruses. Thus, decreasing the cap-binding protein complex either by cleavage of eIF4GI and eIF4GII or by sequestration of eIF4E selectively inhibits translation of capped host cell mRNAs without inhibiting the translation of picornaviral mRNAs. Léonard *et al.* (2004) found that viral protein linked to genome (VPg) of *Turnip mosaic potyvirus* (TuMV) interacts *in vitro* with translation eukaryotic translation initiation factor (eIF) 4E and also showed direct interaction between VPg-Pro (a precursor of VPg) and PABP and between VPg-Pro and eIF4E. Association of PABP with eIF4F results in circularization of mRNAs. Thus, interaction between VPg-Pro and eIF4E as well as PABP could possibly promote viral RNA circularization during translation.

Gallie and Browning (2001) propose that eIF4F (a higher order protein complex that also contains eIF4E besides eIF4G) may promote translation under cellular conditions when cap-dependent translation is inhibited. Viral infection often leads to cap-dependent inhibition of host mRNA translation (Gale *et al.*, 2000; Bushell and Sarnow, 2002). Léonard *et al.* (2004) suggest that TuMV infection of *Brassica pervirdis* leads to inactivation of eIF4E or to its monopolization for viral protein synthesis.

Second method is by substitution of PABP as performed by rotaviruses of *Reoviridae*. Interaction between PABP and eIF4G is disrupted in rotavirus-infected

cells by viral protein NSP3 - leading to reduced efficiency of host mRNA translation and circularization-mediated translational enhancement of rotavirus mRNAs. This also demonstrates how viral nonpolyadenylated mRNAs can usurp the host cell translation apparatus by encoding a protein that binds to the 3`-ends of viral mRNAs, evicting PABP from eIF4G, which is the most important factor involved in recruitment of ribosomes to mRNAs.

Third method is by bypassing of initiator tRNA as practiced by cricket paralysis-like viruses. In *Cricket paralysis virus*, the intergenic region (IGR)-internal ribosome entry site (IRES) sequences themselves occupy the ribosomal P-site; a CCU triplet at the start site base pairs directly with upstream IGR-IRES sequences. This IRES can recruit both 40S and 60S subunits without any known canonical eIFs to form an 80S ribosome that can start protein synthesis from the next codon, a GCU, which is located in the ribosomal A-site. Thus, the first amino acid in the protein is alanine encoded by the A-site located GCU codon. Above findings show that IGR-IRES element propels the ribosome into elongation mode without prior formation of a peptide bond.

V. CLASSIFICATION AND NOMENCLATURE

The various reports of the International Committee on Taxonomy of Viruses (ICTV) have removed much of the confusion and controversy about classification and nomenclature of viruses. In fact, ICTV has evolved a universal system of virus classification on the basis of principles and rules agreed upon by all branches of virology (Mayo, 2002). Its latest report is the Seventh Report published in 2000 (Van Regenmortel *et al.*, 2000) while Mayo (2002) and Van Regenmortel (2002) have summarized the relevant fields of plant viruses. The number of plant virus genera is given below on the basis of their genome types as per Van Regenmortel *et al.* (2000) as mentioned by Mayo (2002): plant virus genera having double-stranded DNA genome - none; plant virus genera having single-stranded DNA genome - 4; plant virus genera having retroid DNA genome - 6; plant virus genera having retroid RNA genome - 2; plant virus genera having double-stranded RNA genome - 6; plant virus genera having single-stranded RNA genome (negative-sense RNA) - 5; plant virus genera having single-stranded RNA genome (positive-sense RNA) - 49; the above numbers together bring the total number of plant virus genera to 72.

A. Classification

The current classification of plant virus genera containing positive-sense RNA genome, shown below, is as per Mayo (2002) [except that the genera have been arranged here in alphabetic order; that the words ‘Unassigned genera’ are included here; and that assignment of various genera to superfamilies, of genera of family *Luteoviridae*, and of ‘Unassigned Genera’ is after Morozov and Solovyev (1999)]. The name given in parenthesis, after the name of genus, is the type species of that particular genus.

1. Family *Bromoviridae* (Alphavirus-like supergroup)
   Genus *Alfamovirus* (*Alfalfa mosaic alfamovirus*)
   Genus *Bromovirus* (*Brome mosaic bromovirus*)
   Genus *Cucumovirus* (*Cucumber mosaic cucumovirus*)
   Genus *Ilarvirus* (*Tobacco streak ilarvirus*)
   Genus *Oleavirus* (*Olive latent oleavirus 2*)
2. Family *Closteroviridae* (Alphavirus-like supergroup)
   Genus *Closterovirus* (*Beet yellows closterovirus*)
   Genus *Crinivirus* (*Lettuce infectious yellows crinivirus*)
3. Family *Comoviridae* (Picorna-like supergroup)
   Genus *Comovirus* (*Cowpea mosaic comovirus*)
   Genus *Fabavirus* (*Broad bean wilt fabavirus*)
   Genus *Nepovirus* (*Tobacco ringspot nepovirus*)
4. Family *Luteoviridae*
   Genus *Enamovirus* (*Pea enation mosaic enamovirus1*) (Sobemo-like supergroup)
   Genus *Luteovirus* (*Barley yellow dwarf luteovirus*-PAV) (Carmo-like supergroup)
   Genus *Polerovirus* (*Potato leafroll polerovirus*) (Sobemo-like supergroup)
5. Family *Potyviridae* (Picorna-like supergroup)
   Genus *Bymovirus* (*Barley yellow mosaic bymovirus*)
   Genus *Ipomovirus* (*Sweet potato mild mottle ipomovirus*)
   Genus *Macluravirus* (*Maclura mosaic macluravirus*)
   Genus *Potyvirus* (*Potato potyvirus Y*)
   Genus *Rymovirus* (*Ryegrass mosaic rymovirus*)
   Genus *Tritimovirus* (*Wheat streak mosaic tritimovirus*)
6. Family *Sequiviridae* (Picorna-like supergroup)
   Genus *Sequivirus* (*Parsnip yellow fleck sequivirus*)
   Genus *Waikavirus* (*Rice tungro spherical waikavirus*)
7. Family *Tombusviridae* (Carmo-like supergroup)
   Genus *Aureusvirus* (*Pothos latent aureusvirus*)
   Genus *Avenavirus* (*Oat chlorotic stunt avenavirus*)
   Genus *Carmovirus* (*Carnation mottle carmovirus*)
   Genus *Dianthovirus* (*Carnation ringspot dianthovirus*)
   Genus *Machlomovirus* (*Maize chlorotic mottle machlomovirus*)
   Genus *Necrovirus* (*Tobacco necrosis necrovirus*)
   Genus *Panicovirus* (*Panicum mosaic panicovirus*)
   Genus *Tombusvirus* (*Tomato bushy stunt tombusvirus*)
8. Unassigned Genera (Genera not assigned to any family by Mayo, 2002)
   Genus *Allexvirus* (*Shallot allexvirus X*) (Family *Potexviridae*)
   Genus *Benyvirus* (*Beet necrotic yellow vein benyvirus*) (Family '*Tubiviridae*')
   Genus *Capillovirus* (*Apple stem grooving capillovirus*) (Proposed order *Tymovirales* of alphavirus-like supergroup)

Genus *Carlavirus* (*Carnation latent carlavirus*) (Family *Potexviridae*)
Genus *Foveavirus* (*Apple stem pitting foveavirus*) (Family *Potexviridae*)
Genus *Furovirus* (*Soil-borne wheat mosaic furovirus*) (Family '*Tubiviridae*')
Genus *Hordeivirus* (*Barley stripe mosaic hodeivirus*) (Family '*Tubiviridae*')
Genus *Idaeovirus* (*Raspberry bushy dwarf idaeovirus*) (Family *Bromoviridae* of alphavirus-like superfamily)
Genus *Marafivirus* (*Maize rayado fino marafivirus*) (Proposed order *Tymovirales* of alphavirus-like supergroup)
Genus *Ourmiavirus* (*Ourmia melon ourmiavirus*)
Genus *Pecluvirus* (*Peanut clump pecluvirus*) (Family '*Tubiviridae*')
Genus *Pomovirus* (*Potato mop-top pomovirus*) (Family '*Tubiviridae*')
Genus *Potexvirus* (*Potato potexvirus X*) (Family *Potexviridae*)
Genus *Sobemovirus* (*Southern bean mosaic sobemovirus*) (Sobemo-like supergroup)
Genus *Tobamovirus* (*Tobacco mosaic tobamovirus*) (Family '*Tubiviridae*')
Genus *Tobravirus* (*Tobacco rattle tobravirus*) (Family '*Tubiviridae*')
Genus *Trichovirus* (*Apple chlorotic leaf spot trichovirus*) (Proposed order *Tymovirales* of alphavirus-like supergroup)
Genus *Tymovirus* (*Turnip yellow mosaic tymovirus*) (Proposed order *Tymovirales* of alphavirus-like supergroup)
Genus *Umbravirus* (*Carrot mottle umbravirus*) (Carmo-like supergroup)
Genus *Vitivirus* (*Grapevine vitivirus A*) (Proposed order *Tymovirales* of alphavirus-like supergroup)

B. Nomenclature

Virologists are strongly opposed to the introduction of Latin binomials for naming viruses (Van Regenmortel, 1989), and the nomenclature system developed by ICTV reflects this position clearly (Van Regenmortel *et al.*, 2000). In formal virus taxonomy, names of orders, families, subfamilies, genera, and species are always printed in italics and the first letters of the names are capitalized, except in case of species where the first letter is also capitalized if they are proper nouns, or parts of proper names. Use of italics for the names of species taxa clearly indicates that the species name has been approved as the official, internationally recognized name (Pringle, 1998). It should be stressed that italics and an initial capital letter need to be used only if the species name refers to a taxonomic category (Van Regenmortel *et al.*, 2000); thus, use of italics is necessary if the name of a species is used as a taxonomic entity.

However, italicized taxonomic names and initial capital letters are not appropriate but the names are written in lower case roman script and correspond to informal vernacular in the following situations. Firstly, when the names refer to physical entities such as the virions found in a virus preparation or seen in an electron micrograph since it is not possible to centrifuge or visualize a virus family (like *Tombusviridae*) or a genus (like *Tobamovirus*), or a species (like *Poliovirus*). Secondly, no italics are

needed when the virus names are used in adjectival form, for instance, tobacco mosaic virus polymerase. Thirdly, when the taxonomic status of a new putative species is not certain or its positioning within an established genus has not been clarified, it is considered as a 'tentative' species and its name is not to be given in italics, although its initial letter will be capitalized.

VI. ABBREVIATIONS

A. Plant Viruses

ACLSV - *Apple chlorotic leafspot trichovirus*
AMV - *Alfalfa mosaic alfamovirus*
ASGV - *Apple stem grooving capillovirus*
BaMV - *Bamboo mosaic potexvirus*
BaYMV - *Barley yellow mosaic bymovirus*
BBMV - *Broad bean mottle bromovirus*
BCMV - *Bean common mosaic bymovirus*
BCV1 - *Beet cryptic alphacryptovirus 1*
BCV3 - *Beet cryptic alphacryptovirus 3*
BlSV - *Bluberry scorch carlavirus*
BMV - *Brome mosaic bromovirus*
BNYVV - *Beet necrotic yellow vein benyvirus*
BrSMV - *Brome streak mosaic rymovirus*
BSMV - *Barley stripe mosaic hordeivirus*
BVQ - *Beet furo-like virus Q*
BWYV - *Beet western yellows luteovirus*
BYDV - *Barley yellow dwarf luteovirus*
BYMV - *Barley yellow mosaic bymovirus*
BYV - *Beet yellows closterovirus*
CaMV - *Cauliflower mosaic caulimovirus*
CarMV - *Carnation mottle carmovirus*
CCMV - *Cowpea chlorotic mottle bromovirus*
CCSV- *Cucumber chlorotic spot closterovirus*
CfMV - *Cocksfoot mottle sobemovirus*
ClYMV - *Clover yellow mosaic potexvirus*
CLRV - *Cherry leafroll nepovirus*
CMV - *Cucumber mosaic cucumovirus*
CNV - *Cucumber necrosis tombusvirus*
CPMV - *Cowpea mosaic comovirus*
CRSV - *Carnation ringspot dianthovirus*
CTLV - *Citrus tatter leaf capillovirus*
CTV - *Citrus tristeza closterovirus*
CymRSV - *Cymbidium ringspot tombusvirus*
GFLV - *Grapevine fanleaf nepovirus*
GRV - *Groundnut rosette umbravirus*
GVA - *Grapevine virus A*
GVB - *Grapevine trichovirus B*
LIYV - *Lettuce infectious yellows closterovirus*
MCMV - *Maize chlorotic mottle machlomovirus*
MFV - *Maize rayado fino virus*
MRDV - *Maize rough dwarf fijivirus*
MStV - *Maize stripe tenuivirus*
OBDV - *Oat blue dwarf marafivirus*
PCV - *Peanut clump pecluvirus*
PDV - *Prune dwarf ilarvirus*
PEBV - *Pea early browning tobravirus*
PEMV - *Pea enation mosaic enamovirus*
PLRV - *Potato leaf roll polerovirus*
PMTV - *Potato mop top pomovirus*
PMV - *Panicum mosaic sobemovirus*
PPV - *Plum pox potyvirus*
PSbMV - *Pea seedborne mosaic potyvirus*
PVM - *Potato carlavirus M*
PVX - *Potato potexvirus X*
PVY - *Potato potyvirus Y*
PYFV - *Parsnip yellow fleck Sequivirus*
RBDV - *Rasberry bushy dwarf idaeovirus*
RCNMV - *Red clover necrotic mosaic dianthovirus*
RDV - *Rice dwarf phytoreovirus*
RGSV - *Rice grassy stunt tenuivirus*
RRSV - *Rice ragged stunt oryzavirus*
RStV - *Rice stripe tenuivirus*
RTSV - *Rice tungro spherical waikavirus*
RYMV - *Rice yellow mottle sobemovirus*
SbDV - *Soybean dwarf luteovirus*
SBMV - *Southern bean mosaic sobemovirus*
SBWMV - *Soil-borne wheat mosaic furovirus*
STNV - *Satellite tobacco necrosis virus*
TBRV - *Tomato black ring nepovirus*
TBSV - *Tomato bushy stunt tombusvirus*
TCV - *Turnip crinkle carmovirus*
TEV - *Tobacco etch potyvirus*
TMV - *Tobacco mosaic tobamovirus*
TNV - *Tobacco necrosis necrovirus*
TomRSV - *Tomato ringspot nepovirus*
TRV - *Tobacco rattle tobravirus*
TSV - *Tobacco streak ilarvirus*
TSWV - *Tomato spotted wilt tospovirus*
TuMV - *Turnip mosaic potyvirus*
TVMV - *Tobacco vein mottling potyvirus*
TYMV - *Turnip yellow mosaic tymovirus*
WClMV - *White clover mosaic potexvirus*
WTV - *Wound tumor phytoreovirus*

B. Other Abbreviations

CI - Cytoplasmic inclusion
CIT - Cap-independent translation
CP - Coat/capsid protein
DI - Defective interfering RNA
ds - Double-strand(ed)
ER - Endoplasmic reticulum
f/s - Frameshift(ing)
HC-Pro - Helper component proteinase
HEL - Helicase
HSP - Heat shock protein
ICR - Internal control region
IRES - Internal ribosome entry site
K/kDa - Kilodalton
MP - Movement protein
MT - Methyltransferase
NI - Nuclear inclusion
NMR - Nuclear magnetic resonance
ns - Non structural
nt - Nucleotide
NTP - Nucleotide triphosphate
NTR - Nontranslated region
ORF(s) - Open reading frame(s)
PABP - Poly(A) binding protein
POL - Polymerase
RdRp - RNA-dependent RNA polymerase
sgRNA - Subgenomic RNA
ss - Single-strand(ed)
TGB - Triple gene block
TLS - tRNA-like structure
UTR - Untranslated region
VPg - Genome-linked protein

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