Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Brome Mosaic Virus

X Wang and P Ahlquist, University of Wisconsin – Madison, Madison, WI, USA

Introduction

Brome mosaic virus (BMV) is a positive-strand RNA virus that infects cereal plants, causing mosaic symptoms and stunting. BMV is the type member of the genus *Bromovirus* in the family *Bromoviridae*, which belong to the alphavirus-like superfamily of human, animal, and plant viruses. BMV has been used as a model for studying gene expression, RNA replication, host–virus interactions, recombination, and encapsidation by positive-strand RNA viruses. Results produced in these areas by many researchers have revealed insights and principles that extend beyond BMV to many other viruses and to general cellular biology. This article reviews selected aspects of this work.

Genome Structure, Expression, and Sequence

In 1971, Lane and Kaesberg used buoyant density gradients to separate BMV virions into three classes having identical capsids but different RNAs (**Figure 1**). Heavy virions contain a single copy of RNA1 (3.2 kbp), medium-density virions contain one copy each of RNA3 (2.1 kbp) and RNA4 (0.9 kbp), and light virions contain a single copy of RNA2 (2.9 kbp). Productive infections require all three virions, but not all four RNAs: infectivity is abolished by omitting RNA1, RNA2, or RNA3, but not RNA4.

Interest in characterizing the protein(s) encoded by each of the four BMV virion RNAs motivated early *in vitro* translation studies with purified BMV virion RNAs. Shih and Kaesberg found that RNAs 1, 2, and 4 are monocistronic while RNA3 is dicistronic. Although early infectivity studies showed that RNA3 encodes coat protein (CP), trypsin degradation analysis showed that the principal translation product of RNA3 was unrelated to CP, while RNA4 served as an excellent template for CP. Moreover, when mixtures of all BMV RNAs were added to wheat germ extracts in increasing amounts, RNA4 inhibited translation of genomic RNAs 1–3. These results showed that RNA4 is a subgenomic CP mRNA derived from RNA3, and implied an elegant system of gene regulation by translational competition: early in infection when viral RNA concentrations are low, all viral proteins including nonstructural RNA replication factors are translated, while after the virion RNAs are sufficiently amplified, CP is preferentially translated to encapsidate these RNAs.

By the early 1970s, RNA bacteriophage studies had provided valuable information on prokaryotic translation initiation sites, including the finding that the first AUG initiation codon usually was 100 or more nucleotides from the RNA 5′-end. In 1975, Dasgupta and Kaesberg characterized the first eukaryotic translation initiation site by isolating two fragments of BMV RNA4 that were efficiently bound by wheat germ ribosomes. RNA sequencing (a challenging, chromatography-based process at the time) revealed that the two fragments were 5′-terminal, overlapping, and encoded the first 4 and 14 amino acids of CP, respectively. The most distinguishing feature was that the initiating AUG codon for CP began only 10 nucleotides (nt) from the $m^7G^{5'}ppp^{5'}$Gp-capped RNA4 5′-end, presaging the now well-known mechanistic linkage between most eukaryotic translation initiation and 5′-mRNA caps.

The ~8.2 kbp BMV genome sequence was completed in 1984. In good agreement with *in vitro* translation results, RNA1 and RNA2 encode single proteins 1a (109 kDa) and 2a (94 kDa), respectively, the 5′-half of RNA3 encodes protein 3a (35 kDa), and the 3′-half of RNA3 encodes RNA4, the subgenomic mRNA for CP (20 kDa) (**Figure 1**). Later work discussed below showed that 3a is required for infection movement in plants. Comparisons with other emerging viral RNA and protein sequences quickly revealed that the BMV 1a and 2a proteins, already implicated in RNA replication by protoplast experiments, shared extensive amino acid sequence similarities with proteins encoded by an outwardly diverse set of plant and animal positive-strand RNA viruses. These similarities initially were recognized between BMV, alfalfa mosaic virus, tobacco mosaic virus, and Sindbis virus, and subsequently were found to extend to many other viruses now grouped together as the alphavirus-like superfamily. Similarities shared by these viruses include a polymerase domain in BMV 2a (hereafter $2a^{pol}$) and RNA capping and RNA helicase-like domains in 1a (**Figure 1**).

Infectious *In Vitro* Transcripts and Foreign Gene Expression

Also in 1984, BMV was used to produce the first infectious transcripts from cloned RNA virus cDNA. Specially designed BMV cDNA clones were transcribed to produce capped *in vitro* transcripts of genomic RNAs 1–3, each with the natural viral RNA 5′-end and only a few extra nucleotides at the 3′-end. Mixtures of all three BMV

RNA transcripts, but not their parent cDNA clones, were infectious to barley plants, a natural BMV host.

This ability to engineer the expression of infectious transcripts provided a means to manipulate the viral RNA genome at the cDNA level using recombinant DNA technology, which has subsequently proved applicable to many other RNA viruses. In one of the first applications of these new reverse genetics approaches, French and co-workers demonstrated that foreign genes could be inserted into the viral genome while retaining the ability to replicate and express genes. Using a transcribable BMV RNA3 cDNA clone, the CP open reading frame (ORF) was replaced with the ORF of the bacterial reporter gene chloramphenicol acetyltransferase (CAT). When *in vitro* transcribed and inoculated onto barley protoplasts with RNA1 and RNA2 transcripts, this RNA3 derivative was replicated and produced CAT activity at higher levels than previously achieved by DNA-based transformation. This first demonstration that RNA viruses can be engineered at the cDNA level showed that the viral RNA genome functions in a sufficiently flexible and modular fashion to tolerate even large changes such as whole gene replacements without substantial optimization, which has significant implications for virus evolution, basic research and biotechnology applications such as development of additional gene expression vectors.

In Vitro and *In Vivo* Replication Studies

Positive-strand RNA viruses like BMV replicate their genomes in a completely RNA-dependent manner, producing a negative-strand RNA replication intermediate for each genomic RNA. Studies on BMV RNA replication were greatly advanced by the development and subsequent use of such tools as *in vitro* RNA-dependent RNA polymerase (RdRp) extracts and cultured plant protoplasts. In 1979, Hall and colleagues developed a virus-specific *in vitro* RdRp extract from BMV-infected barley leaves that synthesized full-length negative-strand RNAs using added BMV virion RNAs as templates. This was the first eukaryotic *in vitro* RdRp preparation exhibiting a high level of template specificity, with other viral RNAs having less than 20% of the template activity of BMV RNAs. As noted in part below, this and similar BMV *in vitro* systems have been utilized by the groups of Hall, Quadt and Jaspars, Kao, and others to make many advances regarding promoters for positive- and negative-strand RNA synthesis, initiation mechanisms, and other issues.

In parallel to *in vitro* systems, cultured plant protoplasts provided a valuable substrate for *in vivo* replication studies due to their ability to be infected or transfected with nearly 100% efficiency with virions, virion RNAs, or *in vitro* transcripts from cloned viral cDNAs. For BMV, barley protoplast systems developed and refined by the groups of Okuno and Furusawa, Hall and others in the late 1970s have allowed studies of all aspects of BMV RNA replication, subgenomic RNA synthesis, progeny RNA encapsidation, and the like. The highly synchronized infections obtained also allowed detailed kinetic studies.

In the early 1990s, it was demonstrated that BMV also would direct RNA replication, subgenomic RNA synthesis, selective viral RNA encapsidation, RNA recombination and the like in the well-studied yeast, *Saccharomyces cerevisiae*. This host provides some of the same advantages as plant protoplasts together with rapid growth, a particularly small genome, well-characterized cell biology, and powerful classical and molecular genetic tools including genome-wide arrays of isogenic yeast strains with each gene systematically modified by deletion, GFP-tagging, etc. Yeast expressing BMV RNA replication factors 1a and $2a^{pol}$ support the replication of BMV genomic RNAs introduced by transfection or DNA-dependent transcription from plasmids or chromosomally integrated expression cassettes, duplicating nearly all features of replication in plant cells. To facilitate yeast genetic studies, BMV RNA replicons can express selectable or screenable markers in yeast and are transmitted to yeast daughter cells during cell division with 85–90% efficiency, rivaling the transmission of yeast DNA plasmids.

BMV Proteins in RNA Replication

Protoplast studies showed that BMV RNA replication and subgenomic RNA4 synthesis require the viral 1a and $2a^{pol}$ proteins but not 3a or CP. As mentioned above, 1a and $2a^{pol}$ share sequence similarity with proteins encoded by other viruses in and beyond the alphavirus-like superfamily. The conserved central domain of $2a^{pol}$ shows similarity to RdRps encoded by picornaviruses and some other RNA viruses. The N-terminal 1a protein domain is related to alphavirus protein nsp1 and contains m^7G methyltransferase and m^7GMP covalent binding activities required for capping viral RNA *in vivo*. The C-terminal 1a domain has sequence similarity to superfamily I NTPase/helicases and NTPase activity that is required for RNA template recruitment and RNA synthesis.

In plant and yeast cells, BMV RNA replication occurs on endoplasmic reticulum (ER) membranes, predominantly in the perinuclear region. In both cell types, 1a localizes to ER membranes in the absence of other viral factors. This localization depends on the N-terminal 1a domain and brings 1a to the cytoplasmic face of ER membranes as a peripheral, not transmembrane, protein. The C-terminal 1a NTPase domain recruits $2a^{pol}$ to ER membranes by interacting with $2a^{pol}$ N-proximal sequences.

In yeast cells replicating BMV RNAs, the ER membrane is modified by numerous 50–60 nm spherular invaginations into the ER lumen. Similar membrane invaginations, designated spherules, are seen in plant cells infected by any of several bromoviruses and in animal cells infected by viruses in and beyond the alphavirus-like superfamily. Immunogold labeling and electron microscopy localized 1a, $2a^{pol}$, and nascent BMV RNAs to the spherule interiors, which remain connected to the cytoplasm by a narrow neck. Protein 1a is sufficient to induce these spherules, and immunogold and biochemical studies show that each spherule contains from one to a few hundred 1a proteins and ~25-fold fewer $2a^{pol}$ proteins. These and other features imply that the structure, assembly, and operation of these spherule replication complexes have functional and perhaps evolutionary links to the replicative cores of retrovirus and dsRNA virus virions, which sequester viral RNA replication templates and their polymerases in a protein shell.

Noncoding Regions and *cis*-Signals

The first region of BMV RNAs to attract attention as a potential regulatory element was the 3′-end. Synergistic work by multiple groups showed that the 3′-noncoding regions of BMV RNAs are highly conserved, multifunctional domains that direct negative-strand RNA synthesis, contribute to RNA encapsidation, translation, and stability, and possess multiple tRNA-like features and functions.

Limited early sequence data showed that BMV RNAs 1–4 share a tRNA-like CCA_{OH} 3′-end. In 1972, Hall and colleagues showed that all four BMV RNAs were selectively aminoacylated *in vitro* with tyrosine. Their further studies showed that tyrosylated BMV RNAs interacted with translation elongation factor 1a and that BMV RNAs were tyrosylated *in vivo* during infection of barley protoplasts. BMV RNA 3′-ends were also found to interact with (ATP, CTP):tRNA nucleotidyl transferase, which can add 3′-CCA_{OH} ends to mature or maintain incomplete BMV RNAs, thus acting as a primitive telomerase. Beginning in the 1970s with work by Dasgupta and Kaesberg, further sequencing, enzymatic structure probing, and three-dimensional computer modeling showed that the 3′ ~200 nt of all BMV RNAs were strongly conserved and folded into an extended, tRNA-like structure with at least two alternate forms that differed in pairing nt near the 3′ with local or distal partners. Similarly conserved, highly structured 3′-regions with related alternate forms were also found in other members of the family *Bromoviridae*.

In vitro and *in vivo* studies using RNA fragments and mutations showed that sequences within the 3′-terminal tRNA-like structure direct negative-strand RNA initiation for RNA replication. Recent results have also implicated the tRNA-like sequence in translation and encapsidation (see below). Involvement in all of these functions led to early and continuing suggestions that the 3′-region mediates co-regulation of the varied uses of BMV positive-strand RNAs to minimize conflicts between multiple essential processes.

A second class of elements combining tRNA-like features, replication signals, and possible interaction with translation are the BMV template recruitment elements, first recognized in RNA3. Deletion analysis revealed that, in addition to 3′- and 5′-sequences required for negative- and positive-strand RNA initiation, BMV RNA3 replication *in vivo* requires a segment of ~150 nt in the 5′-half of the intergenic noncoding region between the 3a and CP ORFs (**Figure 1**). Subsequent studies

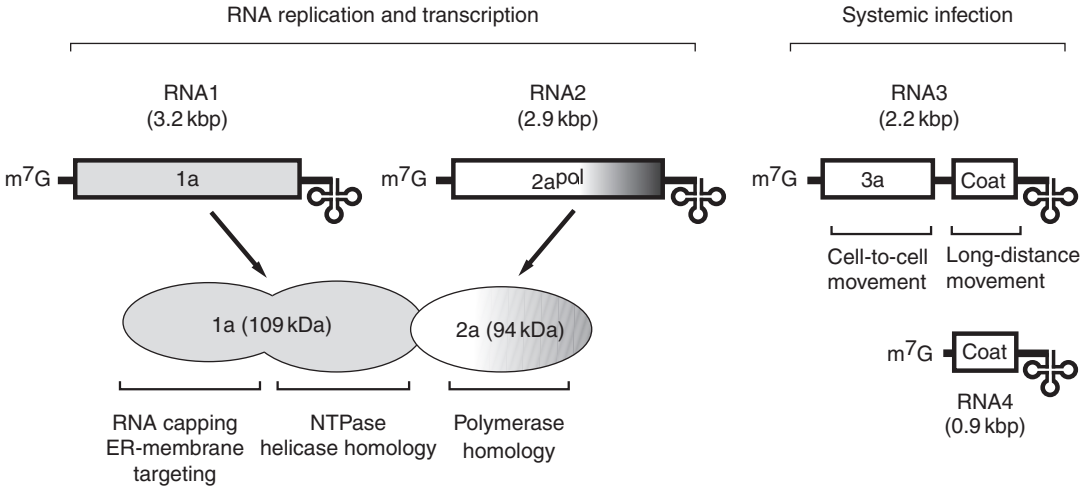

Figure 1 Organization of the brome mosaic virus (BMV) RNA genome. BMV has three genomic RNAs, RNA1, RNA2, and RNA3, and a subgenomic mRNA, RNA4, encoded by the 3′-portion of RNA3 as shown. Each of these four BMV positive-strand RNAs bears a 5′ m^7G cap and 3′ tRNA-like structure (cloverleaf). Open boxes represent the ORFs for the viral proteins 1a and $2a^{pol}$, which are required for RNA replication, and 3a and CP, which are required for systemic movement in host plants. Some specific functions and features of each viral protein are listed.

showed that this region is required for a step prior to negative-strand RNA synthesis, and is necessary and sufficient for 1a to recognize and recruit an RNA to a membrane-associated, translationally inaccessible, nuclease-resistant state that appears to be the spherule interior. Structure probing studies show that this intergenic RNA3 sequence folds into a long, bulged stem loop, which presents at its apex the invariant sequence and structure of tRNA TΨC stem loops. In plant and yeast cells, the appropriate BMV residues in this consensus are modified to T and Ψ, showing that, like the 3′-end, this sequence interacts *in vivo* with tRNA-specific enzymes. Moreover, any mutations to this TΨC stemloop mimicry abolish 1a-mediated template recruitment. Similar stem loops with apical TΨC stem loop regions are found at the extreme 5′-ends of BMV RNA1 and RNA2, where they similarly direct 1a-mediated template recruitment.

As one important aspect of *cis*-signals in BMV replication, the Kao group in particular has defined and dissected minimal core promoters for negative-strand, positive-strand, and subgenomic RNA synthesis, using a variety of approaches. Among other results, their mutational studies imply that the BMV RdRp–promoter interaction has the characteristics of an induced fit, wherein the RdRp has some tolerance to adjust its binding to a range of promoter variants as long as some key sequence features remain. This model potentially reconciles the specificity of BMV RNA synthesis with the ability of the RdRp to synthesize different forms of viral RNA from separate promoters with distinct primary sequences and secondary structures. Kao and associates further found that DNA or DNA/RNA hybrid templates containing the key BMV promoter sequences can be recognized *in vitro* by BMV RdRp extracts and copied into RNA. Although the efficiency of copying DNA templates is ~15-fold lower than for BMV RNA templates, these results have significant potential implications for virus evolution.

Subgenomic mRNA Synthesis

Early observations that the nature of CP in BMV infections is dictated by RNA3 rather than RNA4, and that RNA4 was regenerated when omitted from BMV inoculum, were partially explained when sequencing revealed that RNA4 was encoded by the 3′-portion of RNA3. Nevertheless, whether RNA4 was produced from RNA3 by cleavage or any of several possible RNA synthesis pathways remained uncertain. In 1984, Miller, Hall, and colleagues showed that a BMV RdRp extract produced subgenomic RNA4 *in vitro* when supplied with negative-strand RNA3 templates, and that the product RNA4 could be labeled by γ-^{32}P-GTP incorporation, demonstrating *de novo* initiation. This first elucidation of a pathway for subgenomic mRNA synthesis appears to provide a meaningful precedent for similar subgenomic mRNA synthesis by many positive-strand RNA viruses, and an important foundation for understanding the diversity of alternate mechanisms that has begun to emerge with the demonstration of distinctly different subgenomic mRNA synthesis pathways used by coronaviruses, nodaviruses, and some other positive-strand RNA viruses.

In vivo and *in vitro* analyses of the BMV subgenomic mRNA promoter have complemented well to reveal a core promoter within the 20 nt immediately upstream of the RNA4 start site, which directs low level but accurate initiation of subgenomic mRNA. *In vivo*, the activity of this core promoter is greatly enhanced by upstream sequences that include an oligo(A) tract of variable, ~16–22 nt length in the viral population as well as upstream, partially conserved repeats of core promoter sequences. The important role of the oligo(A) tract suggests that while negative-strand ssRNA can serve as a subgenomic mRNA template *in vitro*, the natural *in vivo* template might be a dsRNA within which the oligo(A) provides a melting site to facilitate internal initiation.

Host Factors in RNA Replication

As for many other viruses, the small size of the 8.2 kbp BMV genome relative to the complexity of BMV replication suggests that many, if not most, steps in BMV RNA replication involve contributions from host factors. Since most of the viral genome is devoted to RNA replication functions, host factors appear particularly likely to be involved in that process as well. Biochemical and genetic results support these ideas and have begun to elucidate some of the relevant host factors and contributions. In 1990, for example, Quadt and Jaspars used anti-1a antiserum to precipitate a complex of BMV 1a, 2a^{pol}, and approximately five host proteins from an active BMV RdRp extract from barley cells. Subsequently, the 41 kDa subunit of cellular translation initiation factor-3 (eIF-3) was found tightly associated with and capable of stimulating BMV RdRp activity.

The ability of BMV to direct RNA replication in yeast further facilitated analysis of host contributions to these processes. Classical yeast genetic approaches identified a number of host genes important to BMV RNA replication, such as *DED1*, a translation factor helicase involved in selective regulation of 2a^{pol} translation; multiple components of the *LSM1–7/PAT1/DHH1* complex, which facilitates recruitment of BMV genomic RNA templates into translation and from translation into RNA replication; *YDJ1*, encoding a chaperone required to activate the RNA replication complex, likely through action on 2a^{pol}; and *OLE1*, encoding a fatty acid desaturase required to produce a membrane lipid profile compatible with membrane-associated viral RNA replication. More

recently, systematic screening of a genome-wide array of yeast knockout strains identified nearly 100 host genes that, when deleted, inhibited or enhanced BMV RNA replication by 3- to >25-fold. These include host genes in RNA, protein, or membrane modification pathways and genes of unknown function, which are shedding further light on BMV replication, virus–host interactions, and cell processes.

RNA Recombination

In 1986, Bujarski and Kaesberg used BMV to provide an early demonstration of RNA recombination in a plant virus. Subsequent work by the Bujarski group and others demonstrated many forms and features of inter- and intramolecular, homologous, and nonhomologous RNA recombination in BMV. Among other findings, Bujarski and collaborators showed that mutations in BMV RNA replication factors 1a and $2a^{pol}$ could alter the frequencies and distributions of crossover sites, implying that at least a significant portion of such recombination was a byproduct of RNA replication, as by template switching. These and other results show that RNA recombination is a major force for repairing BMV genomes damaged by the high mutation rates of viral RNA replication and other events, thereby contributing to BMV survival and adaptability.

Virion Structure and RNA Encapsidation

BMV forms nonenveloped virions ~28 nm in diameter. The outer capsid is composed of 180 copies of CP arranged with $T = 3$ quasi-icosahedral symmetry. Cryo-electron microscope reconstructions by Baker and collaborators and subsequently X-ray crystallography by McPherson and co-workers showed that the BMV capsid structure is extremely similar to that of the related bromovirus, cowpea chlorotic mottle virus (CCMV), whose capsid structure was solved by Johnson and colleagues in 1995. Intriguingly, some features of these capsids are dissimilar from other known isometric RNA virus capsids, but similar to capsids of the DNA-containing papovaviruses. These features include orientation of the core β-barrel nearly perpendicular to the capsid surface to form distinctively prominent hexameric and pentameric capsomeres, and linking of adjacent capsomere clusters by exchange of invading C-terminal arms. These shared features suggest that BMV CP and the CPs of polyoma- and papillomaviruses likely share a common ancestor. BMV virion RNA is arranged as an interior subshell inside the capsid, leaving a hollow virion center. The N-terminal 26 amino acids of the CP, which are highly basic and required for RNA packaging, interact with the RNA to neutralize its charge.

In addition to the predominant 180-subunit capsid, BMV CP can also assemble *in vivo* into a 120-subunit capsid, composed of 60 CP dimers, first discovered in yeast and subsequently confirmed in infected plants. This assembly polymorphism is controlled *in vivo* by the RNA packaged, with BMV RNA2 packaged in 180-subunit capsids, while a small chimeric mRNA containing the CP ORF as its only BMV sequence is packaged in 120-subunit capsids. Structural features shared by 120- and 180-subunit capsids imply that a common pentamer of CP dimers is an important intermediate in BMV virion assembly.

In vitro and *in vivo* encapsidation studies by the Rao group and by Mise, Okuno, Furusawa, and colleagues identified portions of the BMV 3a coding region whose deletion blocked RNA3 encapsidation and interfered with normal co-encapsidation of RNA3 and RNA4. Studies by the Rao group also implicated the 3′ tRNA-like structure of BMV RNAs as a facilitator of encapsidation in *cis* or *trans*, possibly by nucleating CP interactions into productive assemblies such as pentamers of dimers, and showed that BMV RNA replication promotes selective encapsidation of viral RNAs, possibly by inducing coupled synthesis of viral RNA and CP in close proximity.

Infection Spread and Host Range

BMV replicates and encapsidates its RNAs in directly inoculated cells from a wide variety of plants, but has a fairly restricted host range for systemic infection of whole plants. The effective host range for BMV infection thus appears to be determined at the level of initiating or sustaining infection spread from the sites of primary infection. Work by De Jong, Mise, Okuno, Furusawa, Rao, their colleagues, and others have elucidated many features of such spread and its viral determinants.

BMV 3a and CP are dispensable for RNA replication but required for systemic spread. Disruption of the 3a gene blocks cell-to-cell movement, limiting infection to individual, directly inoculated cells. The 3a protein shares multiple properties with cell-to-cell movement proteins of other viruses, including cooperative binding to single-stranded RNA, localization to the plasmodesmatal connections, and induction of virion-containing tubules from the surface of BMV-infected protoplasts. Disruption of the CP gene stops virus spread to noninoculated leaves. Whether local cell-to-cell spread occurs in the absence of CP depends on several factors including the 3a allele and the host plant.

Exchanging genomic RNAs, individual genes, and gene segments among BMV strains and between BMV and other viruses shows that adaptation for infection spread in particular host plants depends not only on 3a

and CP but also on features of RNA1 and RNA2. Host adaptation of 3a generally exerts the predominant effects on infection spread, and only a few amino acid changes in 3a are required to extend BMV host range from monocotyledonous to dicotyledonous plants. However, changes modulating the efficiency of systemic spread also map to 1a and $2a^{pol}$. Such changes may alter systemic spread through host-specific effects on RNA replication, as by influencing the ability of the virus to replicate and spread faster than host defense responses. Alternatively, 1a and $2a^{pol}$ may possess additional functions, as for some C-terminal $2a^{pol}$ sequences that are dispensable for RNA replication but required for efficient systemic infection.

Future Perspectives

Through a variety of intrinsic features and the work of many investigators, research on BMV not only has advanced understanding of bromoviruses, but also has contributed significantly to general virology and molecular biology. Some of the challenges for the future include improved definition and analysis of distinct substeps in viral RNA synthesis including initiation, elongation, termination, and capping; better characterization at molecular, cellular, and tissue levels of the pathways and mechanisms involved in infection spread and the interplay of virus-directed processes and host defenses; and improved understanding of the linkages between different infection processes, including regulated gene expression, RNA replication, encapsidation, and spread.

See also: Alfalfa Mosaic Virus; Bromoviruses; Cucumber Mosaic Virus; *Ilarvirus*; Recombination.

Further Reading

Bujarski JJ and Kaesberg P (1986) Genetic recombination between RNA components of a multipartite plant virus. *Nature* 321: 528–531.

Dreher TW, Bujarski JJ, and Hall TC (1984) Mutant viral RNAs synthesized *in vitro* show altered aminoacylation and replicase template activities. *Nature* 311: 171–175.

French R, Janda M, and Ahlquist P (1986) Bacterial gene inserted in an engineered RNA virus: Efficient expression in monocotyledonous plant cells. *Science* 231: 1294–1297.

Hardy SF, German TL, Loesch-Fries LS, and Hall TC (1979) Highly active template-specific RNA-dependent RNA polymerase from barley leaves infected with brome mosaic virus. *Proceedings of the National Academy of Sciences, USA* 76: 4956–4960.

Johnson JE and Speir JA (1997) Quasi-equivalent viruses: A paradigm for protein assemblies. *Journal of Molecular Biology* 269: 665–675.

Lane LC and Kaesberg P (1971) Multiple genetic components in bromegrass mosaic virus. *Nature New Biology* 232: 40–43.

Lucas RW, Larson SB, and McPherson A (2002) The crystallographic structure of brome mosaic virus. *Journal of Molecular Biology* 317: 95–108.

Miller WA, Dreher TW, and Hall TC (1985) Synthesis of brome mosaic virus subgenomic RNA *in vitro* by internal initiation on (–)-sense genomic RNA. *Nature* 313: 68–70.

Nagano H, Okuno T, Mise K, and Furusawa I (1997) Deletion of the C-terminal 33 amino acids of cucumber mosaic virus movement protein enables a chimeric brome mosaic virus to move from cell to cell. *Journal of Virology* 71: 2270–2276.

O'Reilly EK and Kao CC (1998) Analysis of RNA-dependent RNA polymerase structure and function as guided by known polymerase structures and computer predictions of secondary structure. *Virology* 252: 287–303.

Rao ALN (2006) Genome packaging by spherical plant RNA viruses. *Annual Review of Phytopathology* 44: 61–87.

Schwartz M, Chen J, Janda M, Sullivan M, den Boon J, and Ahlquist P (2002) A positive-strand RNA virus replication complex parallels form and function of retrovirus capsids. *Molecular Cell* 9: 505–514.

Shih DS and Kaesberg P (1976) Translation of the RNAs of brome mosaic virus: The monocistronic nature of RNA1 and RNA2. *Journal of Molecular Biology* 103: 77–88.

Siegel R, Bellon L, Beigelman L, and Kao CC (1999) Use of DNA, RNA and chimeric templates by a viral RNA-dependent RNA polymerase: Evolutionary implications for the transition from the RNA to the DNA world. *Journal of Virology* 73: 6424–6429.

Bromoviruses

J J Bujarski, Northern Illinois University, DeKalb, IL, USA and Polish Academy of Sciences, Poznan, Poland

Introduction

The family *Bromoviridae* represents one of the most important families of plant viruses, infecting a wide range of herbaceous plants, shrubs, and trees. Several of them are responsible for major epidemics in crop plants. The *Bromoviridae* include the spherical icosahedral viruses with a tripartite positive-sense RNA genome. Since these viruses usually accumulate to a high level in the infected tissue, they have been a convenient subject of molecular studies. The type members of different genera, such as cucumber mosaic virus (CMV), brome mosaic virus (BMV), and alfalfa mosaic virus (AMV), constitute excellent molecular models for basic research on viral gene expression, RNA replication, virion assembly, and the role of cellular genes in basic virology.

The genus *Bromovirus* (**Table 1**) comprises not only the best-characterized RNA viruses of the family such as