

FUNCTIONS OF THE 3'-UNTRANSLATED REGIONS OF POSITIVE STRAND RNA VIRAL GENOMES

Theo W. Dreher

Department of Microbiology and Center for Gene Research and Biotechnology, Oregon State University, Corvallis, Oregon 97331-3804; e-mail: dreher@bcc.orst.edu

Key Words RNA replication, translation, tRNA-like structure, poly(A) tail

■ **Abstract** Positive strand RNA viral genomes are unique in the viral world in serving a dual role as mRNA and replicon. Since the origin of the minus-strand RNA replication intermediate is at the 3'-end of the genome, the 3'-untranslated region (UTR) clearly plays a role in viral RNA replication. The messenger role of this same RNA likely places functional demands on the 3'-UTR to serve roles typical of cellular mRNAs, including the regulation of RNA stability and translation. Current understanding indicates varied roles for positive strand RNA viral 3'-UTRs, with the dominant roles differing between viruses. Three case studies are discussed: turnip yellow mosaic virus RNA, whose 3' tRNA mimicry is thought to negatively regulate minus strand synthesis; brome mosaic virus, whose 3'-UTR contains a unique promoter element directing minus strand synthesis; and tobacco mosaic virus, whose 3'-UTR contains an enhancer of translational expression.

INTRODUCTION

The 3'-untranslated regions (UTR) of positive strand viral RNAs were the first segments of these genomes to be sequenced, and have since been a focus of interest and attention from virologists. Among the eukaryotic positive strand RNA viruses, the variety of terminal structures is far greater than that of their hosts' mRNAs, almost all of which are polyadenylated. The occurrence of unusual terminal structures, such as the tRNA-like elements found on some plant viral genomes, has been one reason for interest in 3'-end function. Another has been the recognition that 3'-ends contain the origin of minus strand synthesis and are thus the presumed site of the appropriate promoter elements that control this synthesis *in cis*.

In acknowledgment that positive strand viral genomes function as both mRNAs and replicons, this chapter aims to discuss viral 3'-end function, with substantial consideration of current understanding of the manifold roles of mRNA 3'-UTRs (15). This is intended to provide an additional perspective to the emphasis on

minus strand promotion that has been well covered in recent reviews (10, 28, 67). This chapter discusses (a) the nature of positive strand viral 3'-termini, (b) the possible roles one might a priori expect for these termini, based on the roles of the 3'-UTRs of mRNAs, as well as on consideration of the fact that the 3'-ends must participate directly in viral amplification, and (c) the experimental support that has been obtained for some of these roles. Because of space constraints, the last section is limited to a detailed discussion of only three viral systems, each revealing distinct 3'-functions. The tRNA-like structure (TLS) of turnip yellow mosaic virus (TYMV) has emerged as a minus strand origin whose use appears to be controlled by a novel specificity mechanism that does not require conventional promoter elements; further, it is proposed that negative regulation of minus strand initiation, mediated through 3'-aminoacylation, is an important feature of the replication cycle. On the other hand, studies with brome mosaic virus (BMV) have emphasized a role for a unique minus strand promoter element within the TLS, as well as a telomeric role in 3'-end maintenance. Finally, studies with tobacco mosaic virus (TMV) have emphasized the role of the 3'-UTR in enhancing translational expression of the genome, a role strongly analogous to that of mRNA poly(A) tails.

VARIETY IN 3'-TERMINAL STRUCTURE

Positive strand viral 3'-termini are either TLSs, poly(A) tails, or some other type of non-TLS heteropolymeric sequence. Although to date no commonalities have been discerned to further classify the latter category, this may be possible as more is learned of the 3'-functions of the various viral genomes.

It is interesting that the three types of 3'-termini are not exclusively clustered in phylogenies based on the sequences of the viral RNA-dependent RNA polymerase (RdRp) (63), although TLSs are restricted to proposed Supergroup 3 viruses, and poly(A) tails are absent from the Supergroup 2 viruses (Figure 1). The 3'-ends of two rather distantly related viruses may be more similar than those of very closely related viruses: e.g. both TYMV and BMV RNAs possess TLSs, while the genomes of their respective close relatives oat blue dwarf marafivirus and alfalfa mosaic virus possess a poly(A) tail and heteropolymeric terminus (Figure 1). Indeed, even among viruses within the tymovirus genus, against the backdrop of highly conserved coding characteristics, there is a wide gradation of tRNA mimicry (46).

These observations indicate that the modular evolution that has strongly shaped the coding regions of positive strand viruses (19) also has involved 3'-ends. This suggests that dissimilar 3'-sequences may serve similar roles, and that, at least for some viruses, 3'-end function is not dominated by specific recognition of the 3'-terminus by virus-encoded proteins. Thus, the evolutionary step between different 3'-structures may not be as large as one might expect, and acquisition of a new type of 3'-end by recombination may not require many adjustments in viral coding regions.

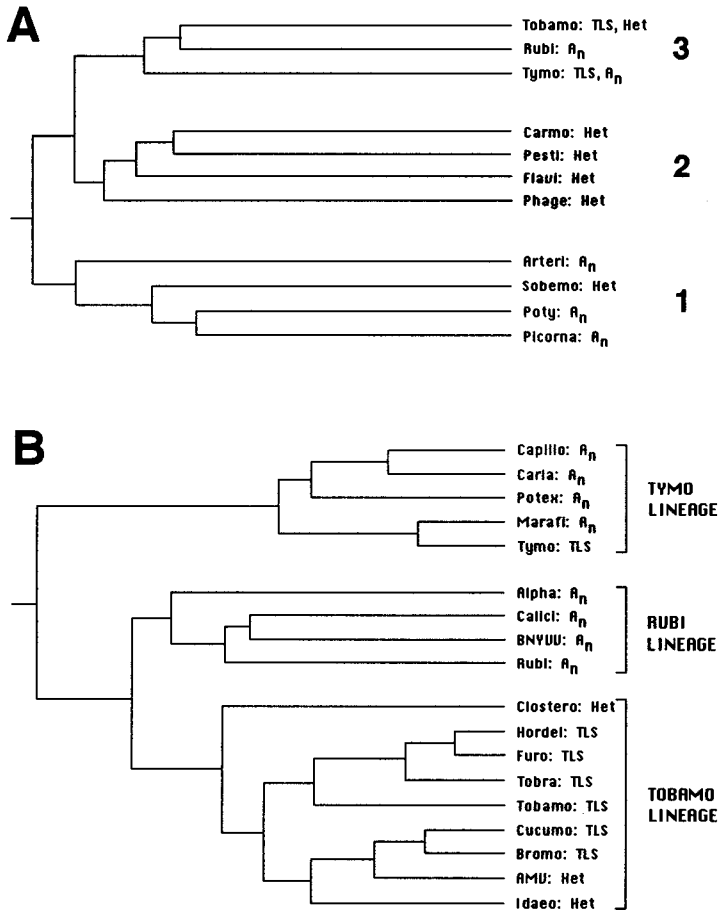


Figure 1 Phylogeny and 3'-UTR. The occurrence of the three classes of 3'-termini found among positive strand RNA viral genomes is presented within the dendrograms that represent a tentative phylogeny of these viruses as proposed by Koonin & Dolja [based on (63)]: TLS, tRNA-like structure; A_n, poly(A) tail; Het, non-TLS heteropolymeric 3'-sequence. *Part A* shows the full phylogeny including the proposed Supergroups 1–3; *Part B* shows genera within Supergroup 3 (63). Branch lengths are approximate and only selected virus groups are shown. BNYVV, beet necrotic yellow vein virus; AMV, alfalfa mosaic virus.

3'-UTRs with tRNA-Like Structures

Transfer RNA-like structures (TLS) are found on the genomes of tymoviruses, several furo-like viruses (46), bromoviruses, cucumoviruses, hordeiviruses, tobamoviruses, and tobnaviruses. The characteristics of the viral TLSs have been reviewed extensively (35, 72), and the discussion here emphasizes new insights and interpretations. The most distinctive characteristic of the viral TLSs is their ability to be aminoacylated in a manner identical to that of tRNAs, catalyzed by specific aminoacyl-tRNA synthetases. Three amino acid specificities are known: valine (tymoviruses, furo-like viruses, and Sunnhemp mosaic tobamovirus), histidine (most tobamoviruses), and tyrosine (bromoviruses, cucumoviruses, and hordeiviruses). The structures of representative TLSs are shown in Figure 2.

Some TLSs have a lower degree of tRNA mimicry and do not support aminoacylation, but are recognized as having a tRNA character by virtue of their 3'-CC derivatives serving as substrates for 3'-adenylation by (CTP, ATP):tRNA nucleotidyltransferase (CCA-NTase). Such TLSs are present in the genomes of tobacco rattle tobnavirus (72) and *erysimum* latent tymovirus (47).

The TLSs of some plant viruses are excellent mimics of tRNA function (35). Recent experiments have emphasized the extremely efficient tRNA mimicry of the valylatable TLSs of the tymoviruses and furo-like viruses. Several TLSs are as efficient substrates for wheat germ valyl-tRNA synthetase and CCA-NTase as is tRNA^{Val}, and form similarly tight ternary complexes with EF-1 α · GTP (21, 27, 46). The efficiency of these properties provides compelling evidence that these functions are relevant *in vivo* and indeed, aminoacylated forms of brome mosaic bromovirus (BMV), barley stripe mosaic hordeivirus (BSMV), (70) and TYMV viral RNAs (57) have been observed *in vivo*.

Surprisingly, against this background of highly efficient tRNA mimicry, the degree of tRNA-like character is not always uniform within a virus genus. For example, among the tymoviral TLSs, two variants of pseudoknotted acceptor/T arms are found, one comprised of 4-, 3-, and 5-basepair helical segments (e.g. TYMV RNA), the other of 3-, 3-, and 6-basepair segments [e.g. eggplant mosaic virus (EMV) RNA]. Both forms support efficient valylation and 3'-adenylation, but the latter type of TLS forms weaker ternary complexes with EF-1 α · GTP (21, 46); it is uncertain whether EMV RNA would compete successfully for EF-1 α binding *in vivo*. A far more divergent, even vestigial, form of TLS is present on ELV RNA. This TLS lacks an anticodon domain and deviates widely from canonical tRNA structure, although there is an acceptor/T arm analogue. The ELV TLS has weak activity as a substrate for 3'-adenylation and cannot be aminoacylated with plant aminoacyl-tRNA synthetases (21, 47).

In the most remarkable case to date, a divergence of tRNA-like properties is found between the two genomic RNAs of peanut clump pecluvirus (PCV), one of the furo-like viruses: RNA1, but not RNA2, can be valylated (46). Although this functional difference is caused by deletion of only a single nucleotide, the resultant difference in 3'-terminal properties is unprecedented among multipartite

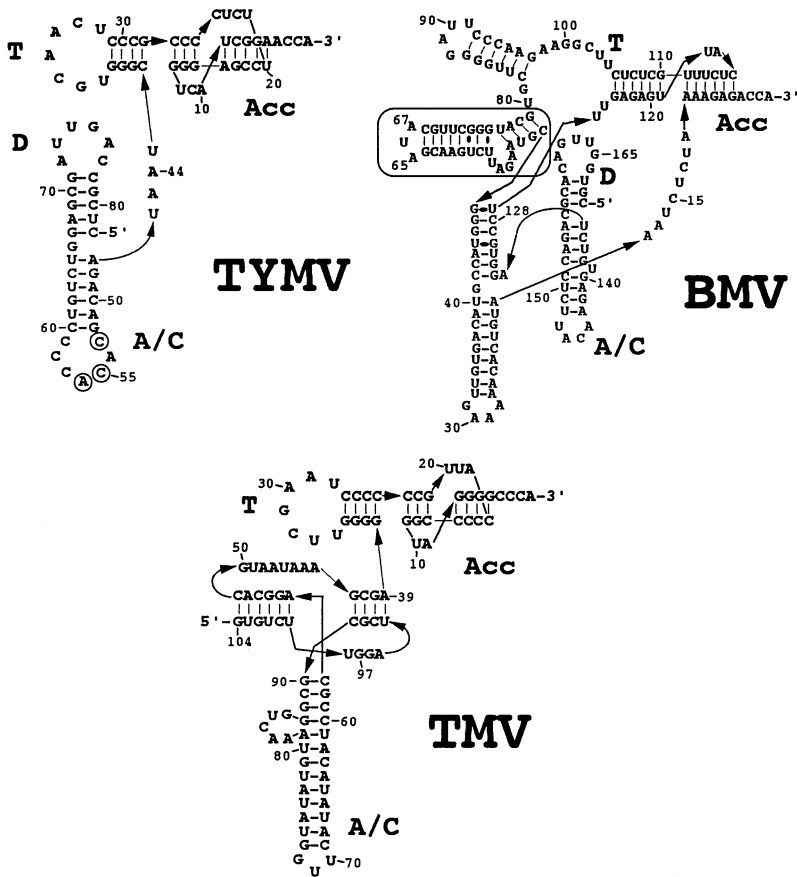


Figure 2 The tRNA-like structures of turnip yellow mosaic, brome mosaic, and tobacco mosaic viral RNAs, which represent the valine, tyrosine, and histidine aminoacylation identities, respectively, found among plant viral RNAs. The secondary structure models were derived from structure-probing experiments (31, 35), and are shown to emphasize overall similarity with tRNAs. The domains analogous to those of canonical tRNAs are indicated for each structure: Acc, aminoacyl acceptor stem; T, T ψ -arm; D, D-arm; A/C, anticodon arm (note that the assignment of the BMV D- and anticodon arms is tentative). The valine identity nucleotides in TYMV RNA (25) are circled, and the proposed RdRp recognition element (13, 20) is boxed in the BMV TLS.

viruses, whose termini are highly conserved (73). The PCV TLS is also of interest in possessing a ~40-nt insertion between the two halves of an otherwise TYMV-like TLS. This insertion is compatible with highly efficient valylation and 3'-adenylation, but is probably responsible for the poor interaction of this valylated TLS with EF-1 α (46).

TLS variety also exists within the tobamoviral genus: Sunnhemp mosaic virus (also known as the cowpea strain of TMV) has a tymoviral-like TLS (21, 75) that is distinct from the typical histidine-specific tobamoviral TLS, and may have been acquired by recombination during a mixed infection with a tymovirus. It has been reported that tomato aspermy cucumoviral RNA is only capable of 3'-adenylation and not capable of accepting tyrosine (56), but another study found that both this RNA and cucumber mosaic cucumoviral RNA could be tyrosylated (33).

While the structural similarity between the valylatable TLSs and canonical tRNAs has permitted an understanding of detailed RNA conformation for some time (35, 72), extended by recent NMR structural studies of the acceptor arm (61), the more complex tobamoviral and bromoviral structures have not been as well understood. Recent structure-probing and modeling studies have refined the proposals for these TLSs (29, 30). These studies have also led to the realization that all the viral TLSs are to some extent substrates for aminoacylation with histidine (32, 94), and that BMV RNA even possesses a low, but detectable, degree of valine identity (31). However, for tymoviral-type TLSs, histidylation by the yeast enzyme requires very high levels of histidyl-tRNA synthetase (21, 46), and the catalytic levels of wheat germ enzyme that are sufficient to aminoacylate TMV RNA or tRNA^{His} are unable to detectably histidylate TYMV and related RNAs (21, 46). Further, 3'-RNA fragments longer than the TLS were substantially poorer substrates for yeast histidyl-tRNA synthetase than the isolated TLS (95). Likewise, the valylation and histidylation of BMV RNA is very inefficient *in vitro* (31). Since it is highly unlikely that the histidylation of BMV and the tymoviral TLSs, and the valylation of BMV RNA, would compete with their primary identities *in vivo*, it seems appropriate to consider these RNAs as possessing a single aminoacylation specificity. Nevertheless, the cryptic aminoacylation identities may assume a physiological role with certain viral mutants.

In many but not all cases, the 3'-UTR harbors a series of one or more nested pseudoknots immediately upstream of the TLS (87). TYMV RNA possesses a single upstream pseudoknot (109) that is, however, not conserved among the tymoviruses (49), whereas TMV and other tobamoviral RNAs possess a linked series of three pseudoknots (110); the existence of the upstream pseudoknots in both of these RNAs is supported by experiments [(49, 109) for TYMV, (68, 110) for TMV].

3'-UTRs with a Poly(A) Tail

Plant viruses whose genomes terminate in poly(A) tails include the potyviruses, potexviruses, comoviruses, capilloviruses, carlaviruses, and beet necrotic yellow vein virus; animal virus representatives include the togaviruses, picornaviruses,

and coronaviruses [tabulated in (10)]. Viral poly(A) tails typically have a length distribution of about 20–100 residues (3, 73), similar to the cytoplasmic length distribution of mRNAs in eukaryotic cells (54).

The structure of the 3'-UTR upstream of the poly(A) tail has been the subject of a number of studies with the enterovirus subgroup of the picornaviruses. Phylogenetic comparisons together with structure-probing experiments (85) led to the proposal that the 3'-UTR features a tertiary structural element formed by base-pairing interactions between the loops of two stem/loops (so-called “kissing” interaction). Mutagenesis experiments with poliovirus (86) and two coxsackie viruses (74, 77) have provided support for this structure rather than an alternative pseudoknotted structure (55) that is also less phylogenetically conserved. Molecular modeling (74, 86) no longer favors the notion that the kissing interaction results in a tRNA-like structural feature, as was previously proposed (85). Nine residues from the poly(A) tail of poliovirus RNA are proposed to be involved in base-pairing to form part of the “kissing” structure (86).

Structure-probing and mutagenesis experiments with bamboo mosaic potexvirus RNA also support the existence of a 3'-structure that includes part of the poly(A) tail, in this case a pseudoknot that incorporates at least 13 adenylate residues (107).

3'-UTRs with Non-TLS Heteropolymeric Terminal Structures

Many viruses possess terminal structures that fit neither of the above two categories. To date, no general commonalities among these terminal structures have been discerned. There are only a few viruses for which there is information on the conformations of these termini and on the types of interactions with proteins these may support. Non-TLS heteropolymeric 3'-termini are found widely among plant viruses, including in sobemo-, luteo-, tombus-, diantho-, clostero-, and alfalfa mosaic viruses, as well as in the coliphages and animal flaviviruses [tabulated in (10)].

Among the best-characterized examples are those from the RNA coliphages (including MS2 and Q β), alfalfa mosaic virus (AMV) and West Nile flavivirus. Secondary structure models for the 3'-terminal domains of the bacteriophage RNAs have been deduced by a combination of ribonuclease S1 structure-probing and phylogenetic covariation analysis (2, 6), revealing a base-paired 3'-terminus adjacent to 3 or 4 stem/loops. It has been considered that the terminal two stems could form an analogue of the acceptor/T arm of canonical tRNAs (2) and perhaps explain the observation that fragmented phage RNA was a substrate for adenylation by CCA-NTase (88). Note, however, that it is not known whether the observed adenylation occurred at the viral 3'-end (88), and it is uncertain whether the bacteriophage RNAs possess any tRNA character. Aminoacylation has not been observed for any coliphage genomic RNA (72).

The 3'-terminal regions of the three genomic RNAs of AMV are highly homologous, and feature five proposed stem/loops flanked by single-stranded (A/U)UGC sequence motifs (111). Ribonuclease structure-probing experiments support the proposed secondary structure, while indicating the presence of alternative

conformations (90). The terminal regions of the AMV RNAs are the site of high-affinity binding by coat protein dimers, an interaction that is necessary in order to establish an infection; this phenomenon has been termed "genome activation," and is shared by AMV and the related ilarviruses (73). For both AMV and the ilarviruses, coat protein or N-terminal coat protein peptides that include a crucial arginine residue (5) bind to RNA elements comprising the basal regions of two adjacent stems flanked by AUGC motifs (4, 51, 64). Covariation within the coat protein binding sites of a number of viruses supports the existence of the stems flanked by (A/U)UGC motifs (4).

Biophysical studies and ribonuclease probing have been used to derive a model for the structure of the 3'-terminal region of West Nile virus RNA (8, 100). The deduced structure comprises a base-paired 3'-terminus and a pseudoknot some 70–90 nt upstream; these features are conserved among the sequenced flavivirus genomes (100).

A PRIORI CONSIDERATION OF POTENTIAL ROLES FOR VIRAL 3'-TERMINI

The genomes of the positive strand RNA viruses are mRNAs, but they are also templates for minus strand RNA synthesis. The properties of their 3'-terminal regions should reflect this dual role. In this section, I consider in turn the functions and attributes such dual roles may be expected to confer on a viral 3'-end, drawing in part from the known functions of cellular mRNAs that have become evident in recent research. These studies have made it clear that the most common site for posttranscriptional regulatory elements is the 3'-UTR, and that elements are varied and versatile (15, 116).

3'-Functions Expected of a Viral Replicon

The 3'-end is clearly the site of important events leading to the onset of the replication of the genome, since minus strands must be synthesized from that terminus. With respect to replication, the 3'-end is not only the origin of minus strand synthesis, but it also could be the site of events that maintain an intact 3'-end.

Minus Strand Promotion The minus strands of those viruses with a 3'-TLS are known from *in vitro* experiments to arise by *de novo* initiation, with insertion of a GTP opposite the 3'-most C residue of the positive strand template (16, 76, 80, 101, 104). The most obvious way to arrange for minus strand synthesis would be for the unique *cis*-active (promoter) elements that control the rate of strand synthesis and that define the site of strand origin to be present adjacent to the site where synthesis begins.

The concept that the main role of the 3'-terminus is to provide a unique binding site for some component of the RdRp complex has probably been the principal

force guiding the design and interpretation of experiments investigating the role of viral 3'-ends. The fact that 3'-termini are conserved among the RNAs of multicomponent viruses appears to support this interpretation; however, sequence conservation is much weaker at the 5'-termini (73), although their complements should, like the various positive strand 3'-ends, each compete successfully for RdRp. The variability of 5'-end sequences could reflect a reliance on specific template selection occurring at the level of minus strand synthesis, followed by coupled positive strand synthesis. On the other hand, it may intimate the existence of alternative mechanisms controlling the origin of RNA synthesis. As a case in point, an internal rather than 3'-terminal RdRp binding site has been implicated in directing template selection by Q β replicase (99 and references therein). A different variation, discovered with TYMV, is discussed in a later section.

Provision of a Telomere A problem peculiar to the replication of linear genetic elements is the avoidance of sequence losses from the termini. For the DNA chromosomes of eukaryotic cells, this problem arises from the dependence of DNA polymerases on RNA primer synthesis by primase. In the case of the positive strand viruses, a similar problem may exist for those viruses that replicate via VPg-primed RNA synthesis (82). Maintenance of 3'-ends is also potentially a problem for all positive strand genomes, due to the action of cellular ribonucleases. It is well known, for example, that the 3'-end—particularly the 3'-terminal A residue—of tRNA turns over in the cytoplasm (18). Cytoplasmic CCA-NTase maintains intact 3'-CCA termini through its adenylation activity [it can replace the entire -CCA triplet if necessary (18)].

Viruses may be expected to utilize cellular end-maintenance activities. Viral genomes with 3'-TLSs have access to cellular CCA-NTase, whose role in 3'-end maintenance is discussed below for BMV. Polyadenylated viral genomes may benefit from host cytoplasmic polyadenylation activities. Although new transcripts are polyadenylated in the nucleus, re-polyadenylation of shortened poly(A) tails can occur in the cytoplasm in some instances, especially during oocyte maturation and in the early development of animal embryos (15, 93). In vertebrate cells, cytoplasmic polyadenylation is controlled jointly by an AAUA(A/U)A element 10–30 nt upstream of the poly(A) tail and a (UU)UUUUAAU-like element (the so-called cytoplasmic polyadenylation element, CPE) less than 60 nt further upstream (93). The full range of signals controlling cytoplasmic polyadenylation is probably not known currently, and it is not known if the plant cells that host viral infections are able to support cytoplasmic polyadenylation of mRNAs.

Perusal of the 3'-UTRs of polyadenylated viral genomic RNAs in GenBank reveals that the two-component U-rich CPE/AAUAAA polyadenylation signals are absent from both plant and animal viral RNAs. The potexviruses appear to be the only virus group with an identifiable potential polyadenylation signal, an AAUAAA or AAUAUA element that is present close to the poly(A) tail. White clover mosaic potexviral RNAs with truncated poly(A) tails are able to produce progeny with normal tail lengths (48), but the enzyme activity responsible for A

addition has not been identified. However, it is intriguing that point mutation of an AAUAAA sequence in the 3'-UTR led to shorter poly(A) tails (48).

Regulation of Access to the Minus Strand Origin A central theme of regulated gene expression in prokaryotic and eukaryotic cells is that the activities of promoters directing DNA-dependent transcription are under many regulatory controls. Analogous control of translational initiation by regulated ribosomal access has been demonstrated for MS2 and other bacteriophages (14). It would seem likely that controlled access to the minus strand RNA origin, including repression, is a feature of the positive strand viral lifecycle. Possible roles for negative regulation would be the coordination of translation and replication functions, which are incompatible on the one RNA molecule (60), or the shut-down of minus strand synthesis that occurs during many animal virus infections and that appears to occur in at least some plant virus infections, as evidenced by early plateaus in the level of minus strands (52, 66).

Packaging Although packaging signals could, in principal, be located anywhere within the viral genomic segments, the presence of conserved sequences only at the 3'-ends of the genomic segments of multipartite viruses such as the bromo-, cucumo-, tobra-, and furo-like viruses suggests that packaging signals may be located near the 3'-ends. As for minus strand promoters and RNA replication, this conclusion derives from the expectation that specific high-affinity recognition sites control encapsidation. There is currently no evidence that encapsidation sites are in fact located within conserved 3'-termini. The encapsidation sites of the two positive strand RNA plant viruses (both monopartite) that have been identified to date are both located toward the 3'-end of the genome, but within coding regions [TMV (73); turnip crinkle virus (89)]; it is not certain whether the 3'-terminal coat protein binding sites of AMV are involved in encapsidation (73).

3'-Functions of Cellular mRNAs That May Pertain to Viral RNAs

RNA Stability The poly(A) tails of mRNAs are known to be intimately involved in the turnover or degradation of mRNAs (15, 54). In tobacco protoplasts, a poly(A) tail stabilized electroporated mRNAs two- to fourfold (37), and for many mRNAs, degradation is preceded by a shortening of the poly(A) tail (54). Poly(A)-binding proteins (PABP) are key players in the metabolism of polyadenylated RNAs. Requiring a minimum of 12 adenosines for binding, they are ubiquitously associated with poly(A) tails, binding in strings with a spacing of about 25 nt (54). Bound PABP can protect mRNAs in vitro against attack by 3'-5' exonucleases (7), but in yeast PABP is also a cofactor in the deadenylation activity of a poly(A) nuclease (71).

Other features within the 3'-UTR influence mRNA stability, in either a positive or negative way (15, 54). AU-rich elements (AREs) are found in the 3'-UTRs of

many mammalian mRNAs that have short half-lives, such as those mRNAs encoding oncoproteins, cytokines, and transcription factors. AREs vary considerably in sequence, but most commonly contain multiple copies of the pentanucleotide AUUUA. A number of proteins that bind to AREs have been reported (54); while some of these interactions are presumably involved in accelerating degradation, they can also protect against cleavage, reflecting a regulated aspect to the rapid mRNA turnover that is specified by AREs (83).

The protection against degradation by bound proteins has also been observed with other mRNAs that possess specific protein binding sites (15). For example, the 3'-UTR of the mammalian transferrin receptor mRNA contains five iron-responsive elements (IREs) that are bound by IRE-binding protein to afford protection against ribonuclease cleavage (59).

Little is known of instability determinants in plant mRNAs (1), but one such determinant has been identified in the 3'-UTR of an auxin-induced mRNA (45). It has also been demonstrated that AREs are able to act as instability signals in plant mRNAs, as evidenced by model studies in which a synthetic AUUUA repeat was placed in the 3'-UTR of two reporter mRNAs (79).

Modulation of Translational Expression In recent years, studies on translational initiation have emphasized the notion that translationally active mRNAs are effectively circularized through a network of interactions mediated by RNA-binding proteins (38, 53, 97, 115). This model arose from *in vitro* and *in vivo* observations indicating that the poly(A) tail plays a direct role in promoting the translational expression of an mRNA (53). Poly(A) tail-dependent stimulation of translation, which acts synergistically with a 5'-cap-mediated stimulation, has been observed in plant cells (37). Translation of circularized mRNAs is considered to provide the benefits of recycling posttermination ribosomal subunits back to the 5'-end of the RNA. Support for such circularization comes from the observation that polysomes in some tissues are circular (38, 53) and from direct demonstration that translation initiation factors can mediate the circularization of an mRNA (115). Current understanding from both yeast and plants is that both PABP bound to the poly(A) tail and eIF4E (eIFiso4E in plants) bound to the 5'-cap directly contact the initiation factor eIF4G (eIFiso4G in plants) (38, 97, 115).

Certain viral RNAs have been found to provide alternative pathways to this general scheme: Picornaviral RNAs are translated in a cap-independent manner, mediated by direct eIF4G binding to the internal ribosome entry site (62, 97), whereas TMV and BMV RNAs possess in their 3'-UTR sequences that functionally substitute for the poly(A) tail (38, 39, 41; discussed in more detail below).

The 3'-UTRs of mRNAs also harbor a variety of elements that serve to negatively regulate, or repress, translation without leading to degradation (15, 116). In the examples characterized to date, such regulation serves to store mRNAs in a sequestered state away from ribosomes until translation is developmentally appropriate. The molecular players involved in at least two instances of translational repression from the 3'-UTR are now known: two hnRNPs that bind to a CU-rich

repetitive sequence motif in erythroid 15-lipoxygenase mRNA (81), and a previously unidentified RNA-binding protein (FBF) that binds to the UCUUG element in the fem-3 sex-determining mRNA of *Caenorhabditis elegans* (117).

Targeting of RNA to Specific Subcellular Sites Some mRNAs within eukaryotic cells have limited, specific subcellular distribution, and are not distributed uniformly throughout the cytoplasm (15, 44). Examples are mRNAs that encode the proteins involved in establishing the positions of body segment boundaries in the developing insect embryo, or actin mRNAs in embryonic muscle cells. Analysis of the *cis*-elements responsible for the specific localization of such mRNAs has invariably indicated the presence of “zip-code” elements in the 3'-UTR. These elements can be functionally transplanted into heterologous mRNAs to direct the same specific localization, and they presumably represent sites for the binding of proteins that are involved in the specific tethering of the mRNAs.

Localization of viral components is a strong theme in the amplification cycle of eukaryotic positive strand RNA viruses: Replication occurs in association with specific cellular membranes [reviewed in (36); e.g. chloroplasts for tymoviruses (69, 73), peroxisomes for most tombusviruses (96)]. Further, plant viral genomes in some form must at one time localize to the plasmodesmata to permit cell-to-cell movement. However, such localizations are not necessarily the results of direct RNA-host protein interactions, but could be the result of protein-protein interactions involving viral protein(s) bound in some way to the RNA genome. Indeed, a region of the ORF1 product is the likely determinant of the specific membrane tropism of tombusviruses (12), and it has been shown that a 6-kDa protein encoded by tobacco etch potyvirus interacts specifically with the endoplasmic reticulum (98).

EXPERIMENTAL SUPPORT FOR ROLES OF VIRAL 3'-TERMINI

Case Study, TYMV: Role of TLS as a Repressor of Minus Strand Synthesis Rather Than Unique Promoter Element?

The role of the TLS has been extensively studied with TYMV, since the structural similarity of the TYMV TLS to tRNA makes this system the most tractable for investigating the role of tRNA mimicry. One approach has been to observe the effects of experimentally modifying the aminoacylation characteristics of the valine-specific TLS. By altering only a few nucleotides, principally in the anticodon loop, it has been possible to partially or almost completely remove the ability to become valylated (25) and to switch the aminoacylation identity from valine to methionine (26). Inoculation studies with these mutants showed that amplification of the viral genome in Chinese cabbage or turnip protoplasts was directly correlated with the efficiency of aminoacylation assayed *in vitro*: RNAs

with progressively diminished valine acceptance amplified to progressively lower levels in protoplasts (108). The strongest mutants resulted in undetectable coat protein or genomic RNA amplification, although some ^{32}P [phosphate] was incorporated into double-stranded genomic RNA (108). A revertant had acquired a second mutation that simultaneously improved infectivity and valylation (109). Genomic RNAs whose identities had been switched to methionine were infectious in plants (26). These experiments have demonstrated that aminoacylatability is an important property for genome amplification, but the amino acid esterified at the 3'-end of the RNA need not be valine.

Other experiments with TYMV involved assessing the infectivity and replication of chimeric genomes in which the TYMV TLS was replaced with other sequences. Viable chimeras resulted when the natural TLS was replaced by structurally similar, valylatable tymoviral TLSs, even though the sequences varied considerably (103). A chimera bearing the 3'-end from ELV RNA, which cannot be aminoacylated (see above) and has strong structural and sequence differences from the TYMV TLS, was infectious (though attenuated) to plants (47). Chimeras with 3'-terminal regions comprising the TLS and clustered upstream pseudoknots from the 3'-UTRs of TMV and BMV amplified to very low levels in protoplasts and were not infectious to plants. Finally, no amplification was detected of genomes bearing a 3'-poly(A) tail or tRNA^{Val} (103). These studies indicate that the main function of the TYMV TLS is other than to act as a generic tRNA-like element, and show that the TYMV 3'-end and remainder of the genome are co-adapted. Importantly, however, the BMV and TMV chimeras demonstrate that there is some flexibility in the type of 3'-end that can be replicated to low levels.

The simplest interpretation of these results is that the 3'-end contains a specific promoter directing minus strand synthesis. However, *in vitro* experiments with a partially purified RdRp preparation made from TYMV-infected Chinese cabbage leaves have not supported this conclusion. Although this enzyme discriminates against non-tymoviral genomic RNAs, it is able to transcribe minus strands efficiently from a range of short tRNA-like RNAs, including the TMV TLS (16, 101). Using the TYMV TLS as a reference, template activity was not affected by deletion of the T-, D-, or anticodon domains (102), and disruptions of the acceptor stem pseudoknot resulted in no more than a twofold reduction in template activity (17, 101). In agreement, TLS fragments containing little more than the pseudoknotted acceptor stem (43), the acceptor stem itself (101) or a truncated stem-loop derivative of the acceptor stem (17) were active templates. Experiments from two laboratories thus indicate the absence of a potent specific promoter of minus strand synthesis within the TLS upstream of the initiation region.

Of course, the TYMV TLS is not devoid of *cis*-acting signals directing minus strand synthesis, but these are amazingly restricted to the -CCA at the 3'-terminus, which acts as a strong initiation box. This property is demonstrated by analyzing as templates RNAs with reiterated -CCA- triplets added to the normal 3'-end of the TLS, or even reiterated -CCA- triplets without any additional upstream sequences: The linear GG(CCA)₁₂ and the TLS-(CCA)_n₆ RNAs are similarly

efficient templates, and multiple -CCA- sites serve for initiation (102). Secondary structure introduced into the GG(CCA)₁₂ template led to selectivity for initiation among the -CCA- potential initiation sites, and base-paired -CCA- triplets were not available for initiation (102). It was concluded that initiation site selection by TYMV RdRp is driven by a strong preference for initiation at -CC(A/G)- sites modulated by structural accessibility, in place of the usual expectation of a unique promoter or enhancer element (67). If this interpretation is correct, one can view an important role of the TLS as presenting the 3'-CCA in a highly accessible conformation, but in a nonspecific way; the efficiency of valylation and EF-1 α binding of the TYMV genomic RNA (21) attests to the exceptional accessibility of the 3'-CCA. At the same time, in view of the fact that the short -CC(A/G)-sequence is present many times within the 6.3-kb genome, other occurrences of this sequence should fall at structurally inaccessible sites. Such a specificity scheme that does not rely on unique controlling sequences or structures (promoter elements) may be assisted by other factors, such as the strong *cis*-preferential replication exhibited by TYMV (113) and many other viruses.

This view of minus strand initiation is compatible with conclusions on the role of tRNA mimicry deduced from studies of novel infectious genomes with altered tRNA-like properties. In addition to the genome with methionine identity (26), three infectious genomes that fail to aminoacylate have been isolated (47). These are based on chimeras with TMV- and ELV-derived 3'-ends, and their infectivity shows that, although aminoacylation is crucial in the context of the normal TYMV TLS, the role of this TLS can be taken over by a terminus that lacks aminoacylation, but presumably possesses some offsetting attribute. If we assume that positive regulation of minus strand synthesis must be mediated by creating some specific signal or by making some specific contact to the viral RdRp, while negative regulation can be mediated by a stand-alone repressor that physically blocks access to the initiation site, the sum of experimental data (47, 103, 108) suggests that tRNA mimicry serves a repressive role. This is argued by the fact that three key properties of the wild-type RNA—the valine identity nucleotides and the interaction with valyl-tRNA synthetase and EF-1 α —can be dispensed with under certain circumstances (all are missing in the 3'-ELV chimera). The negative regulator for wild-type TYMV RNA would almost certainly be EF-1 α , which binds tightly precisely over the 3'-initiation site; for those infectious mutant genomes that fail to aminoacylate and so do not bind EF-1 α , another host protein presumably acts as repressor. Negative regulation of minus strand synthesis may serve to delay the switch from translation to replication, or promote a switch from negative to positive strand synthesis.

Is TYMV a special case in possessing an RdRp with minimal sequence constraints for minus strand synthesis and a postulated negative regulation of minus strand availability? These properties have permitted the TYMV proteins to amplify genomes with quite different 3'-ends that were provided by experimental recombination. The recombinational shuffling of 3'-ends that appears to have occurred during the evolution of modern viruses (Figure 1) suggests that these

properties may not be unique to TYMV among the positive strand RNA viruses.

Case Study, BMV: Role of TLS as a Telomere and Site of Specific Promoter Elements

The role of the TLS has also been intensively investigated for BMV. The experiments showing that the TLS comprises the promoter of minus strand synthesis have heavily influenced the field. As with TYMV, minus strand synthesis initiates *de novo* opposite the 3'-most C in the 3'-CCA terminus (58, 76). 3'-RNA fragments as short as 134 nts are excellent templates (76). Many mutations within this fragment that alter the secondary or tertiary structure decrease template activity (11, 22), but activity is also severely diminished by single point mutations within the -A₆₇U₆₆- loop sequence and by deletion of a bulge loop in the subtending stem (22) (see Figure 2). These mutations do not affect tyrosylation or 3'-adenylation of the RNA with CCA-NTase (20, 23), suggesting that the stem that includes the A₆₇U₆₆A₆₅ loop sequence and the bulge loop constitutes a specific minus strand promoter element. This has recently been confirmed by *in vitro* RdRp experiments in which short RNAs containing the above loop sequence and subtending stem were strong inhibitors of minus strand synthesis from 3'-BMV RNA fragments; further, these RNAs could themselves be converted into active templates by addition of a 3'-ACCA initiation site (13). RNAs with point mutations in the AUA loop sequence or deletion of the bulge were neither inhibitors of other templates nor active templates themselves when provided with a 3'-ACCA. It is not known which protein is responsible for recognition of this apparently specific promoter element.

Because of the complexity of the BMV TLS and strong divergence from the structure of canonical tRNA, the precise tRNA-mimicking elements other than the acceptor/T arm have not been elucidated with certainty. For instance, although detailed structure-probing, tyrosyl-tRNA synthetase interaction and modeling studies have led to revised proposals for the structure of the BMV TLS and have identified putative anticodon-like domains (29, 84), these assignments have not been experimentally verified. Studies on the role of tRNA mimicry have thus been hampered by uncertainty regarding the location of tyrosine identity elements. Nevertheless, small mutations specifically diminishing the tyrosylation but not 3'-adenylation or minus strand template activities were identified (23) and used to assess the role of aminoacylation *in vivo*. When present on BMV RNA3, such mutations only slightly decreased virus amplification in barley protoplasts (24), but when present on RNA2 (92) or RNA1 (28), these RNAs failed to amplify. These results suggest that aminoacylation is crucial *in cis* for RNAs 1 and 2, which encode essential replication proteins, but not for RNA3, which is replicated *in trans*. Although it is possible that the limited mutations used in these studies were defective in some other essential property, it appears that the amplification of BMV RNAs 1 and 2, and of TYMV RNA, are similarly dependent on aminoacylation. Due to the different effect of defective aminoacylation on the three BMV RNAs, it is unlikely

that aminoacylation is involved in promoting minus strand synthesis, a conclusion that was also reached for TYMV (see above).

Experiments with BMV have convincingly shown that the TLS can serve a telomeric role, probably through 3'-end maintenance by host CCA-NTase. A two-nucleotide substitution mutation in the RNA3 TLS that specifically diminished CCA-NTase-dependent 3'-adenylation resulted in the loss of one or two 3'-nucleotides from some RNA3 progeny, although the wild-type termini of RNAs 1 and 2 were intact (24). In other experiments, RNA3s with mutant minus strand initiation sites (substitutions in the 3'-CA) showed near-normal amplification kinetics in barley protoplasts, and progeny RNAs had regenerated wild-type termini (91). It was concluded that, as with tRNAs, the 3'-terminal nucleotides of BMV RNA experience turnover, and restoration, presumably by host CCA-NTase acting as a telomerase, is important in maintaining intact termini.

Case Study, TMV: Role of Upstream Pseudoknots in Enhancing Translational Expression

The most significant emphasis of studies on the role of the TMV 3'-UTR has been on its role as a *cis* element capable of enhancing translational expression. In translational expression studies in protoplasts, the TMV 3'-UTR can functionally replace the 3'-poly(A) of GUS or luciferase reporter mRNAs in both monocot and dicot cells (41, 68). The main contribution to the enhanced expression for 5'-capped RNAs is from improved translational efficiency, which increased eightfold in tobacco protoplasts (68) and 24- to 39-fold in carrot protoplasts (39); the 3'-UTR and 5'-cap contributed synergistically to this effect. A smaller contribution to increased expression in the presence of the TMV 3'-UTR derived from increased mRNA stability (1.5- to threefold longer half-life; 39, 68), an effect that was independent of the 5'-cap. These effects of the TMV 3'-UTR on translational efficiency, RNA stability, and synergism with the 5'-cap were roughly equivalent to the effects of a poly(A) tail (41, 68). In parallel studies, the BMV 3'-UTR was also shown to promote translational expression, though to a slightly lower extent than the TMV 3'-UTR, whereas the TYMV and alfalfa mosaic virus 3'-termini did not stimulate translation (39).

The increased translational expression induced by the TMV 3'-UTR is principally due to the pseudoknot tract upstream of the TLS (37, 41). The role of the TLS itself as a translational modulator is unresolved. All constructs used in experiments have had nonnative 3'-ends (37, 41) that would have prevented aminoacylation and EF-1 α interaction. Nevertheless, the presence of the TMV TLS did improve RNA stability and expression levels about twofold (37, 41), an effect that was lost when the 3'-terminus was truncated within the acceptor stem (37). Removal of the TLS from its position adjacent to the upstream pseudoknot tract also resulted in a roughly twofold decrease in expression (37, 41).

Extensive mutational studies have indicated that the effect of the upstream pseudoknot tract is mainly due to sequences conserved among tobamoviral genomes

that fall within the downstream-most of the three pseudoknots (68). Structure-disrupting mutations decreased the translational enhancement, but compensatory mutations failed to identify a particular base-paired element that was required for the enhancement. The same pseudoknot was shown to include sequences essential for infectivity in plants and replication in protoplasts (105). In this latter study, base-pairing was required in the downstream stem segment of the pseudoknot, but base-pairs could be reversed. Neither study (68, 105) resolved whether a pseudo-knotted conformation is in fact required, and it is not known whether there is a connection between the role of this part of the 3'-UTR in translational enhancement and in determining infectivity.

The role of the upstream pseudoknot tract as a translational enhancer has been probed further by identifying a 102-kDa binding protein that also binds to another translational enhancer [the Ω element (40)] that is present in the 5'-UTR of TMV RNA; binding is tighter to Ω than to the 3'-UTR (106). The binding protein is a member of the HSP101/HSP104/ClpB family of heat shock proteins whose expression in yeast recapitulated the translational enhancement of the Ω and 3'-UTR elements, which are not active in wild-type yeast (114). This translational enhancement is codependent on at least two translation initiation factors (eIF4G and eIF3). It may be envisioned that binding of the HSP101 to both 5' and 3' enhancers and a direct or indirect interaction with initiation factors results in a circularization of the RNA much like that described earlier for mRNAs with poly(A) tails (38).

This scenario described for the TMV 3'-UTR may be similar with barley stripe mosaic hordeivirus (BSMV) RNA, but with a twist. In the BSMV RNAs, there is a short poly(A) tract immediately upstream of the TLS (65). The 15- to 43-nucleotide poly(A) length reported for various BSMV isolates in GenBank is sufficient for association with the poly(A) binding protein, which could lead to RNA circularization from the analogous internal 3'-UTR site as deduced for TMV RNA.

CONCLUDING REMARKS

The three case studies discussed present clear indications of varied viral 3'-end function, in line with the wide spectrum of properties directed by the 3'-UTRs of cellular mRNAs (15). It seems certain that future research will uncover more and varied roles. It has been a goal of this review to stimulate the adoption of a wide consideration of possible roles when interpreting and designing experiments on viral 3'-UTR function. Several host proteins that interact with the 3'-ends of viral genomes have been identified (67), and roles for some of these may well parallel mRNA 3'-function rather than being directly involved in promoting minus strand synthesis. Correct assignment of the role of a binding protein or of a 3'-UTR *cis*-element can be difficult, since several types of defects may most obviously manifest themselves as a defect in RNA replication. For instance, a defect in translation will

not be distinguishable in an inoculation experiment from a defect in minus strand initiation, necessitating the design and use of appropriate specialized assays.

A second conclusion emerging from the case studies is that while some functions may be shared by 3'-UTRs of different viruses, others may be specialized. The variety of 3'-UTR functions, and the ways in which different viruses implement and emphasize shared functions, is likely to be large. Some viral RNAs possess unique minus strand promoter elements (e.g. BMV), whereas only small initiation boxes are identifiable in others (e.g. TYMV); the extent to which *cis*-preferential replication (or coupled translation and replication) exists for a given virus (78, 113) is likely to influence the type of minus strand selectivity mechanism. Those viruses with polyadenylated genomes presumably achieve translational efficiency through a functional circularization involving the poly(A) tail, while TMV does the same with a translational enhancer in the upstream pseudoknot tract. The translational enhancer in the 3'-UTR of PAV barley yellow dwarf virus (112) may represent another variation of this theme.

Future research on a broad front will bring fresh understanding of the interconnected roles of the 3'-UTR, and might lead to explanations for the observation that some positive strand viruses possess (interrupted) complementarity between their 5'- and 3'-termini (34). We should learn whether the role of genome activation of the alfalfa mosaic virus coat protein (73) is a minus strand repression function like that postulated above for EF-1 α in TYMV replication. And we should come to understand better the switch from the translation to the RNA replication phase of a viral infection (42, 60).

Visit the Annual Reviews home page at <http://www.AnnualReviews.org>

LITERATURE CITED

1. Abler M, Green P. 1996. Control of mRNA stability in higher plants. *Plant Mol. Biol.* 32:63-78
2. Adhin MR, Alblas J, van Duin J. 1990. Secondary structure at the 3' terminal region of RNA coliphages: comparison with tRNA. *Biochim. Biophys. Acta* 1050:110-8
3. Ahlquist P, Kaesberg P. 1979. Determination of the length distribution of poly(A) at the 3' terminus of the virion RNAs of EMC virus, poliovirus, rhinovirus, RAV-61 and CPMV and of mouse globin mRNA. *Nucleic Acids Res.* 7:1195-204
4. Ansel-McKinney P, Gehrke L. 1998. RNA determinants of a specific RNA-coat protein peptide interaction in alfalfa mosaic virus: conservation of homologous features in ilarvirus RNAs. *J. Mol. Biol.* 278:767-85
5. Ansel-McKinney P, Scott SW, Swanson M, Ge X, Gehrke L. 1996. A plant viral coat protein RNA binding consensus sequence contains a crucial arginine. *EMBO J.* 15:5077-84
6. Beekwilder MJ, Nieuwenhuizen R, van Duin J. 1995. Secondary structure model for the last two domains of single-stranded RNA phage Q beta. *J. Mol. Biol.* 247:903-17
7. Bernstein P, Peltz SW, Ross J. 1989. The poly(A)-poly(A)-binding protein complex is a major determinant of mRNA stability in vitro. *Mol. Cell. Biol.* 9:659-70
8. Brinton MA, Fernandez AV, Dispoto JH. 1986. The 3'-nucleotides of flavivirus

- genomic RNA form a conserved secondary structure. *Virology* 153:113–21
9. Brinton MA, Rueckert RR, eds. 1987. *Positive Strand RNA Viruses*. New York: Liss
 10. Buck KW. 1996. Comparison of the replication of positive-stranded RNA viruses of plants and animals. *Adv. Virus Res.* 47:159–251
 11. Bujarski JJ, Dreher TW, Hall TC. 1985. Deletions in the 3'-terminal tRNA-like structure of brome mosaic virus RNA differentially affect aminoacylation and replication in vitro. *Proc. Natl. Acad. Sci. USA* 82:5636–40
 12. Burgyan J, Rubino L, Russo M. 1996. The 5'-terminal region of a tombusvirus genome determines the origin of multi-vesicular bodies. *J. Gen. Virol.* 77:1967–74
 13. Chapman M, Kao CC. 1999. A minimal RNA promoter for minus-strand RNA synthesis by the Brome Mosaic Virus polymerase complex. *J. Mol. Biol.* 286:709–20
 14. de Smit MH, van Duijn J. 1990. Control of prokaryotic translational initiation by mRNA secondary structure. *Prog. Nucleic Acid Res. Mol. Biol.* 38:1–35
 15. Decker CJ, Parker R. 1995. Diversity of cytoplasmic functions for the 3' untranslated region of eukaryotic transcripts. *Curr. Opin. Cell Biol.* 7:386–92
 16. Deiman BA, Koenen AK, Verlaan PW, Pleij CW. 1998. Minimal template requirements for initiation of minus-strand synthesis in vitro by the RNA-dependent RNA polymerase of turnip yellow mosaic virus. *J. Virol.* 72:3965–72
 17. Deiman BA, Kortlever RM, Pleij CW. 1997. The role of the pseudoknot at the 3' end of turnip yellow mosaic virus RNA in minus-strand synthesis by the viral RNA-dependent RNA polymerase. *J. Virol.* 71:5990–96
 18. Deutscher M. 1982. tRNA Nucleotidyltransferase. In *The Enzymes* 15:183–215. Academic
 19. Dolja V, Carrington J. 1992. Evolution of positive-strand RNA viruses. *Semin. Virol.* 3:315–26
 20. Dreher TW, Bujarski JJ, Hall TC. 1984. Mutant viral RNAs synthesized in vitro show altered aminoacylation and replicase template activities. *Nature* 311:171–75
 21. Dreher TW, Goodwin JB. 1998. Transfer RNA mimicry among tymoviral genomic RNAs ranges from highly efficient to vestigial. *Nucleic Acids Res.* 26:4356–64
 22. Dreher TW, Hall TC. 1988. Mutational analysis of the sequence and structural requirements in brome mosaic virus RNA for minus strand promoter activity. *J. Mol. Biol.* 201:31–40
 23. Dreher TW, Hall TC. 1988. Mutational analysis of the tRNA mimicry of brome mosaic virus RNA. Sequence and structural requirements for aminoacylation and 3'-adenylation. *J. Mol. Biol.* 201:41–55
 24. Dreher TW, Rao AL, Hall TC. 1989. Replication in vivo of mutant brome mosaic virus RNAs defective in aminoacylation. *J. Mol. Biol.* 206:425–38
 25. Dreher TW, Tsai CH, Florentz C, Giegé R. 1992. Specific valylation of turnip yellow mosaic virus RNA by wheat germ valyl-tRNA synthetase determined by three anticodon loop nucleotides. *Biochemistry* 31:9183–89
 26. Dreher TW, Tsai CH, Skuzeski JM. 1996. Aminoacylation identity switch of turnip yellow mosaic virus RNA from valine to methionine results in an infectious virus. *Proc. Natl. Acad. Sci. USA* 93:12212–16
 27. Dreher TW, Uhlenbeck OC, Browning K. 1999. Quantitative assessment of EF-1 α -GTP binding to aminoacyl-tRNA, aminoacyl-viral RNA and tRNA shows close correspondence to the RNA binding properties of EF-Tu. *J. Biol. Chem.* 274:666–72
 28. Duggal R, Lahser F, Hall T. 1994. Cis-acting sequences in the replication of plant

- viruses with plus-sense RNA genomes. *Annu. Rev. Phytopathol.* 32:287–309
29. Felden B, Florentz C, Giegé R, Westhof E. 1994. Solution structure of the 3'-end of brome mosaic virus genomic RNAs. Conformational mimicry with canonical tRNAs. *J. Mol. Biol.* 235:508–31
 30. Felden B, Florentz C, Giegé R, Westhof E. 1996. A central pseudoknotted three-way junction imposes tRNA-like mimicry and the orientation of three 5' upstream pseudoknots in the 3' terminus of tobacco mosaic virus RNA. *RNA* 2:201–12
 31. Felden B, Florentz C, Westhof E, Giegé R. 1998. Transfer RNA identity rules and conformation of the tyrosine tRNA-like domain of BMV RNA imply additional charging by histidine and valine. *Biochem. Biophys. Res. Commun.* 243:426–34
 32. Felden B, Giegé R. 1998. Resected RNA pseudoknots and their recognition by histidyl-tRNA synthetase. *Proc. Natl. Acad. Sci. USA* 95:10431–36
 33. Fernandez-Cuartero B, Burgyan J, Aranda MA, Salanki K, Moriones E, Garcia-Arenal F. 1994. Increase in the relative fitness of a plant virus RNA associated with its recombinant nature. *Virology* 203:373–77
 34. Florentz C, Briand J, Giegé R. 1984. Possible functional role of viral tRNA-like structures. *FEBS Lett.* 176:295–300
 35. Florentz C, Giegé R. 1995. tRNA-like structures in plant viral RNAs. In *tRNA: Structure, Biosynthesis and Function*, ed. D Söll, UL RajBhandary, pp. 141–63. Washington, DC: ASM Press
 36. Francki R. 1987. Responses of plant cells to virus infection with special reference to sites of RNA replication. See Ref. 9, pp. 423–36
 37. Gallie DR. 1991. The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes Dev.* 5:2108–16
 38. Gallie DR. 1998. A tale of two termini: a functional interaction between the termini of an mRNA is a prerequisite for efficient translation initiation. *Gene* 216:1–11
 39. Gallie DR, Kobayashi M. 1994. The role of the 3'-untranslated region of non-polyadenylated plant viral mRNAs in regulating translational efficiency. *Gene* 142:159–65
 40. Gallie DR, Sleat DE, Watts JW, Turner PC, Wilson TM. 1987. The 5'-leader sequence of tobacco mosaic virus RNA enhances the expression of foreign gene transcripts in vitro and in vivo. *Nucleic Acids Res.* 15:3257–73
 41. Gallie DR, Walbot V. 1990. RNA pseudoknot domain of tobacco mosaic virus can functionally substitute for a poly(A) tail in plant and animal cells. *Genes Dev.* 4:1149–57
 42. Gamarnik A, Andino R. 1998. Switch from translation to RNA replication in a positive-stranded RNA virus. *Genes Dev.* 12:2293–304
 43. Gargouri-Bouزيد R, David C, Haenni AL. 1991. The 3' promoter region involved in RNA synthesis directed by the turnip yellow mosaic virus genome in vitro. *FEBS Lett.* 294:56–58
 44. Gavis E. 1997. Expeditions to the pole: RNA localization in *Xenopus* and *Drosophila*. *Trends Cell Biol.* 7:485–92
 45. Gil P, Green P. 1996. Multiple regions of the Arabidopsis SAUR-AC1 gene control transcript abundance: the 3' untranslated region functions as an mRNA instability determinant. *EMBO J.* 15:1678–86
 46. Goodwin JB, Dreher TW. 1998. Transfer RNA mimicry in a new group of positive-strand RNA plant viruses, the furoviruses: differential aminoacylation between the RNA components of one genome. *Virology* 246:170–78
 47. Goodwin JB, Skuzeski JM, Dreher TW. 1997. Characterization of chimeric turnip yellow mosaic virus genomes that are infectious in the absence of aminoacylation. *Virology* 230:113–24

48. Guilford PJ, Beck DL, Forster RL. 1991. Influence of the poly(A) tail and putative polyadenylation signal on the infectivity of white clover mosaic potyvirus. *Virology* 182:61–67
49. Hellendoorn K, Mat AW, Gulyaev AP, Pleij CW. 1996. Secondary structure model of the coat protein gene of turnip yellow mosaic virus RNA: long, C-rich, single-stranded regions. *Virology* 224:43–54
50. Hershey JWB, Mathews MB, Sonenberg N, eds. 1996. *Translational Control*. Cold Spring Harbor: Cold Spring Harbor Lab. Press
51. Houser-Scott F, Ansel-McKinney P, Cai JM, Gehrke L. 1997. In vitro genetic selection analysis of alfalfa mosaic virus coat protein binding to 3'-terminal AUGC repeats in the viral RNAs. *J. Virol.* 71:2310–19
52. Ishikawa M, Meshi T, Ohno T, Okada Y. 1991. Specific cessation of minus-strand RNA accumulation at an early stage of tobacco mosaic virus infection. *J. Virol.* 65:861–68
53. Jacobson A. 1996. Poly(A) metabolism and translation: the closed-loop model. See Ref. 50, pp. 451–80
54. Jacobson A, Peltz SW. 1996. Interrelationships of the pathways of mRNA decay and translation in eukaryotic cells. *Annu. Rev. Biochem.* 65:693–739
55. Jacobson SJ, Konings DA, Sarnow P. 1993. Biochemical and genetic evidence for a pseudoknot structure at the 3' terminus of the poliovirus RNA genome and its role in viral RNA amplification. *J. Virol.* 67:2961–71
56. Joshi R, Haenni AL. 1986. Search for tRNA-like properties in tomato aspermy virus RNA. *FEBS Lett.* 194:57–94
57. Joshi S, Chapeville F, Haenni AL. 1982. Turnip yellow mosaic virus RNA is aminoacylated in vivo in Chinese cabbage leaves. *EMBO J.* 1:935–38
58. Kao CC, Sun JH. 1996. Initiation of minus-strand RNA synthesis by the brome mosaic virus RNA-dependent RNA polymerase: use of oligoribonucleotide primers. *J. Virol.* 70:6826–30
59. Klausner RD, Rouault TA, Harford JB. 1993. Regulating the fate of mRNA: the control of cellular iron metabolism. *Cell* 72:19–28
60. Kolakofsky D, Weissmann C. 1971. Possible mechanism for transition of viral RNA from polysome to replication complex. *Nature New Biol.* 231:42–46
61. Kolk MH, van der Graaf M, Wijmenga SS, Pleij CW, Heus HA, Hilbers CW. 1998. NMR structure of a classical pseudoknot: interplay of single and double-stranded RNA. *Science* 280:434–38
62. Kolupaeva VG, Pestova TV, Hellen CU, Shatsky IN. 1998. Translation eukaryotic initiation factor 4G recognizes a specific structural element within the internal ribosome entry site of encephalomyocarditis virus RNA. *J. Biol. Chem.* 273:18599–604
63. Koonin EV, Dolja VV. 1993. Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences. *Crit. Rev. Biochem. Mol. Biol.* 28:375–430
64. Koper-Zwarthoff EC, Bol JF. 1980. Nucleotide sequence of the putative recognition site for coat protein in the RNAs of alfalfa mosaic virus and tobacco streak virus. *Nucleic Acids Res.* 8:3307–18
65. Kozlov YV, Rupasov VV, Adyshev DM, Belgelarskaya SN, Agranovsky AA, et al. 1984. Nucleotide sequence of the 3'-terminal tRNA-like structure in barley stripe mosaic virus genome. *Nucleic Acids Res.* 12:4001–9
66. Kroner PA, Young BM, Ahlquist P. 1990. Analysis of the role of brome mosaic virus 1a protein domains in RNA replication, using linker insertion mutagenesis. *J. Virol.* 64:6110–20
67. Lai MM. 1998. Cellular factors in the

- transcription and replication of viral RNA genomes: a parallel to DNA-dependent RNA transcription. *Virology* 244:1–12
68. Leathers V, Tanguay R, Kobayashi M, Galie DR. 1993. A phylogenetically conserved sequence within viral 3' untranslated RNA pseudoknots regulates translation. *Mol. Cell. Biol.* 13:5331–47
 69. Lesemann D. 1977. Virus group-specific and virus-specific cytological alterations induced by members of the tymovirus group. *Phytopathol. Z.* 90:315–21
 70. Loesch-Fries LS, Hall TC. 1982. In vivo aminoacylation of brome mosaic and barley stripe mosaic virus RNAs. *Nature* 298:771–73
 71. Lowell JE, Rudner DZ, Sachs AB. 1992. 3'-UTR-dependent deadenylation by the yeast poly(A) nuclease. *Genes Dev.* 6:2088–99
 72. Mans RM, Pleij CW, Bosch L. 1991. tRNA-like structures. Structure, function and evolutionary significance. *Eur. J. Biochem.* 201:303–24
 73. Matthews REF. 1991. *Plant Virology*. San Diego: Academic. 3rd ed.
 74. Melchers WJ, Hoenderop JG, Bruins Slot HJ, Pleij CW, et al. 1997. Kissing of the two predominant hairpin loops in the coxsackie B virus 3' untranslated region is the essential structural feature of the origin of replication required for negative-strand RNA synthesis. *J. Virol.* 71:686–96
 75. Meshi T, Ohno T, Iba H, Okada Y. 1981. Nucleotide sequence of a cloned cDNA copy of TMV (cowpea strain) RNA, including the assembly origin, the coat protein cistron, and the 3' non-coding region. *Mol. Gen. Genet.* 184:20–25
 76. Miller WA, Bujarski JJ, Dreher TW, Hall TC. 1986. Minus-strand initiation by brome mosaic virus replicase within the 3' tRNA-like structure of native and modified RNA templates. *J. Mol. Biol.* 187:537–46
 77. Mirmomeni MH, Hughes PJ, Stanway G. 1997. An RNA tertiary structure in the 3' untranslated region of enteroviruses is necessary for efficient replication. *J. Virol.* 71:2363–70
 78. Novak JE, Kirkegaard K. 1994. Coupling between genome translation and replication in an RNA virus. *Genes Dev.* 8:1726–37
 79. Ohme-Takagi M, Taylor C, Newman T, Green P. 1993. The effect of sequences with high AU content on mRNA stability in tobacco. *Proc. Natl. Acad. Sci. USA* 90:11811–15
 80. Osman TA, Buck KW. 1996. Complete replication in vitro of tobacco mosaic virus RNA by a template-dependent, membrane-bound RNA polymerase. *J. Virol.* 70:6227–34
 81. Ostareck DH, Ostareck-Lederer A, Wilm M, Thiele BJ, Mann M, Hentze MW. 1997. mRNA silencing in erythroid differentiation: hnRNP K and hnRNP E1 regulate 15-lipoxygenase translation from the 3' end. *Cell* 89:597–606
 82. Paul AV, van Boom JH, Filippov D, Wimmer E. 1998. Protein-primed RNA synthesis by purified poliovirus RNA polymerase. *Nature* 393:280–84
 83. Peng SS, Chen CY, Xu N, Shyu AB. 1998. RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. *EMBO J.* 17:3461–70
 84. Perret V, Florentz C, Dreher T, Giegé R. 1989. Structural analogies between the 3' tRNA-like structure of brome mosaic virus RNA and yeast tRNA^{Tyr} revealed by protection studies with yeast tyrosyl-tRNA synthetase. *Eur. J. Biochem.* 185:331–39
 85. Pilipenko EV, Maslova SV, Sinyakov AN, Agol VI. 1992. Towards identification of cis-acting elements involved in the replication of enterovirus and rhinovirus RNAs: a proposal for the existence of tRNA-like terminal structures. *Nucleic Acids Res.* 20:1739–45
 86. Pilipenko EV, Poperechny KV, Maslova

- SV, Melchers WJ, Slot HJ, Agol VI. 1996. Cis-element, oriR, involved in the initiation of (-) strand poliovirus RNA: a quasi-globular multi-domain RNA structure maintained by tertiary ('kissing') interactions. *EMBO J.* 15:5428-36
87. Pleij CWA, Abrahams JP, van Belkum A, Rietveld K, Bosch L. 1987. The spatial folding of the 3' noncoding region of aminoacylatable plant viral RNAs. See Ref. 9, pp. 299-316
88. Prochiantz A, Benicourt C, Carre D, Haenni AL. 1975. tRNA nucleotidyltransferase-catalyzed incorporation of CMP and AMP into RNA-bacteriophage genome fragments. *Eur. J. Biochem.* 52:17-23
89. Qu F, Morris TJ. 1997. Encapsulation of turnip crinkle virus is defined by a specific packaging signal and RNA size. *J. Virol.* 71:1428-35
90. Quigley GJ, Gehrke L, Roth DA, Auron PE. 1984. Computer-aided nucleic acid secondary structure modeling incorporating enzymatic digestion data. *Nucleic Acids Res.* 12:347-66
91. Rao AL, Dreher TW, Marsh LE, Hall TC. 1989. Telomeric function of the tRNA-like structure of brome mosaic virus RNA. *Proc. Natl. Acad. Sci. USA* 86:5335-39
92. Rao AL, Hall TC. 1991. Interference in trans with brome mosaic virus replication by RNA-2 bearing aminoacylation-deficient mutants. *Virology* 180:16-22
93. Richter J. 1996. Dynamics of poly(A) addition and removal during development. See Ref. 50, pp. 481-503
94. Rudinger J, Felden B, Florentz C, Giegé R. 1997. Strategy for RNA recognition by yeast histidyl-tRNA synthetase. *Bioorg. Med. Chem.* 5:1001-9
95. Rudinger J, Florentz C, Dreher T, Giegé R. 1992. Efficient mischarging of a viral tRNA-like structure and aminoacylation of a minihelix containing a pseudoknot: histidinylation of turnip yellow mosaic virus RNA. *Nucleic Acids Res.* 20:1865-70
96. Russo M, Di Franco A, Martelli GP. 1983. The fine structure of Cymbidium ringspot virus infections in host tissues. III. Role of peroxisomes in the genesis of multivesicular bodies. *J. Ultrastruct. Res.* 82:52-63
97. Sachs AB, Sarnow P, Hentze MW. 1997. Starting at the beginning, middle, and end: translation initiation in eukaryotes. *Cell* 89:831-38
98. Schaad MC, Jensen PE, Carrington JC. 1997. Formation of plant RNA virus replication complexes on membranes: role of an endoplasmic reticulum-targeted viral protein. *EMBO J.* 16:4049-59
99. Schuppli D, Miranda G, Qiu S, Weber H. 1998. A branched stem-loop structure in the M-site of bacteriophage Q β RNA is important for template recognition by Q β replicase holoenzyme. *J. Mol. Biol.* 283:585-93
100. Shi PY, Brinton MA, Veal JM, Zhong YY, Wilson WD. 1996. Evidence for the existence of a pseudoknot structure at the 3' terminus of the flavivirus genomic RNA. *Biochemistry* 35:4222-30
101. Singh RN, Dreher TW. 1997. Turnip yellow mosaic virus RNA-dependent RNA polymerase: initiation of minus strand synthesis in vitro. *Virology* 233:430-39
102. Singh RN, Dreher TW. 1998. Specific site selection in RNA resulting from a combination of nonspecific secondary structure and -CCR- boxes: initiation of minus strand synthesis by turnip yellow mosaic virus RNA-dependent RNA polymerase. *RNA* 4:1083-95
103. Skuzeski JM, Bozarth CS, Dreher TW. 1996. The turnip yellow mosaic virus tRNA-like structure cannot be replaced by generic tRNA-like elements or by heterologous 3' untranslated regions known to enhance mRNA expression and stability. *J. Virol.* 70:2107-15
104. Sun JH, Adkins S, Faurote G, Kao CC. 1996. Initiation of (-)-strand RNA

- synthesis catalyzed by the BMV RNA-dependent RNA polymerase: synthesis of oligonucleotides. *Virology* 226:1–12
105. Takamatsu N, Watanabe Y, Meshi T, Okada Y. 1990. Mutational analysis of the pseudoknot region in the 3' noncoding region of tobacco mosaic virus RNA. *J. Virol.* 64:3686–93
 106. Tanguay RL, Gallie DR. 1996. Isolation and characterization of the 102-kilodalton RNA-binding protein that binds to the 5' and 3' translational enhancers of tobacco mosaic virus RNA. *J. Biol. Chem.* 271:14316–22
 107. Tsai C-H, Cheng C-P, Peng C-W, Lin B-Y, Lin N-S, Hsu Y-H. 1999. Sufficient length of a poly(A) tail for the formation of a potential pseudoknot is required for efficient replication of bamboo mosaic potexvirus RNA. *J. Virol.* 73:2703–9
 108. Tsai CH, Dreher TW. 1991. Turnip yellow mosaic virus RNAs with anticodon loop substitutions that result in decreased valylation fail to replicate efficiently. *J. Virol.* 65:3060–67
 109. Tsai CH, Dreher TW. 1992. Second-site suppressor mutations assist in studying the function of the 3' noncoding region of turnip yellow mosaic virus RNA. *J. Virol.* 66:5190–99
 110. van Belkum A, Abrahams JP, Pleij CW, Bosch L. 1985. Five pseudoknots are present at the 204 nucleotides long 3' noncoding region of tobacco mosaic virus RNA. *Nucleic Acids Res.* 13:7673–86
 111. van Rossum CM, Brederode FT, Neeleman L, Bol JF. 1997. Functional equivalence of common and unique sequences in the 3' untranslated regions of alfalfa mosaic virus RNAs 1, 2, and 3. *J. Virol.* 71:3811–16
 112. Wang S, Browning K, Miller W. 1997. A viral sequence in the 3'-untranslated region mimics a 5' cap in facilitating translation of uncapped mRNA. *EMBO J.* 16:4107–16
 113. Weiland JJ, Dreher TW. 1993. Cis-preferential replication of the turnip yellow mosaic virus RNA genome. *Proc. Natl. Acad. Sci. USA* 90:6095–99
 114. Wells DR, Tanguay RL, Le H, Gallie DR. 1998. HSP101 functions as a specific translational regulatory protein whose activity is regulated by nutrient status. *Genes Dev.* 12:3236–51
 115. Wells SE, Hillner PE, Vale RD, Sachs AB. 1998. Circularization of mRNA by eukaryotic translation initiation factors. *Mol. Cell* 2:135–40
 116. Wickens M, Kimble J, Strickland S. 1996. *Translational control of developmental decisions*. See Ref. 50, I. 411–50
 117. Zhang B, Gallegos M, Puoti A, Durkin E, Fields S, et al. 1997. A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. *Nature* 390:477–84



CONTENTS

Reflections on Space, Time, and Diversity, <i>JC Zadoks</i>	1
George Henry Hepting: Pioneer Leader in Forest Pathology, <i>Ellis B. Cowling, Arthur Kelman, Harry R. Powers Jr.</i>	19
Freedom to Operate: Intellectual Property Protection in Plant Biology and Its Implications for the Conduct of Research, <i>Janice A. Kimpel</i>	29
Crown Gall of Grape: Biology and Disease Management, <i>Thomas J. Burr, Leon Otten</i>	53
The Three Ds of PCR-Based Genomic Analysis of Phytobacteria: Diversity, Detection and Diagnosis, <i>FJ Louws, JLW Rademaker, FJ de Bruijn</i>	81
Effects of Plants on Nematode Community Structure, <i>GW Yeates</i>	127
Functions of the 3'-Untranslated Regions of the Positive Strand RNA Viral Genomes, <i>Theo W. Dreher</i>	151
Polyketide Production by Plant-Associated <i>Pseudomonas</i> , <i>CL Bender, V Rangaswamy, J Loper</i>	175
The Evolution of Asexual Fungi: Reproduction, Speciation, and Classification, <i>JW Taylor, DJ Jacobson, MC Fisher</i>	197
The <i>Caenorhabditis elegans</i> Genome: A Guide in the Post-Genomic Age, <i>David McK Bird, Charles H Opperman, Steven JM Jones, David L Baillie</i>	247
Taxonomy and Identification of <i>Septoria</i> and <i>Stagonospora</i> Species on Small Grain Cereals, <i>Barry M Cunfer, Peter P Ueng</i>	267
Phytoalexins: What We Have Learned After 60 Years?, <i>Ray Hammerschmidt</i>	285
Withholding and Exchanging Iron: Interactions between <i>Erwinia</i> spp. and Their Plant Hosts, <i>D Expert</i>	307
THE TOMATO- <i>CLADOSPORIUM FULVUM</i> INTERACTION: A Versatile Experimental System to Study Plant-Pathogen Interactions, <i>MHAJ Joosten, PJGM de Wit</i>	335
Natural Genomic and Antigenic Variation in Whitefly- Transmitted Geminiviruses (Begomoviruses), <i>BD Harrison, DJ Robinson</i>	369
Climate Change and Plant Disease Management, <i>Stella Melugin Coakley, Harald Scherm, Sukumar Chakraborty</i>	399
Biocontrol Agents Within the Context of Soil Microbial Communities: A Substrate-Dependent Phenomenon, <i>HAI Hoitink, MJ Boehm</i>	427
The Dark Side of the Mycelium: Melanins of Phytopathogenic Fungi, <i>Joan M Henson, Michael J Butler, Alan W Day</i>	447
Host Variation for Interactions with Beneficial Plant- Associated Microbes, <i>Kevin P Smith, Robert M Goodman</i>	473