

Translation in plants – rules and exceptions

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Abstract

Translation processes in plants are very similar to those in other eukaryotic organisms and can in general be explained with the scanning model. Particularly among plant viruses, unconventional mRNAs are frequent, which use modulated translation processes for their expression: leaky scanning, translational stop codon readthrough or frameshifting, and transactivation by virus-encoded proteins are used to translate polycistronic mRNAs; leader and trailer sequences confer (cap-independent) efficient ribosome binding, usually in an end-dependent mechanism, but true internal ribosome entry may occur as well; in a ribosome shunt, sequences within an RNA can be bypassed by scanning ribosomes. Translation in plant cells is regulated under conditions of stress and during development, but the underlying molecular mechanisms have not yet been determined. Only a small number of plant mRNAs, whose structure suggests that they might require some unusual translation mechanisms, have been described.

Introduction

Plant cells harbor three different translation machineries: the eukaryotic ribosomes in the cytoplasm and systems for translation in mitochondria and plastids. The

transcriptional and post-transcriptional regulation of chloroplast gene expression has been reviewed recently [233, 282]. This review deals exclusively with translation events and mRNAs in the cytoplasm and concentrates on *cis*-active sequences that influence efficiency

and fidelity of translation. Although the translation of only a few nuclear plant genes has been analyzed so far, it is very safe to assume that translation of plant mRNAs in general proceeds according to the rules established for other eukaryotic systems. The 'exceptions' described mostly apply to viral RNAs encoded by cytoplasmic RNA viruses or by plant pararetroviruses in the nucleus.

The rules

Most of the different translation processes have been elaborated in mammalian cell systems, in extracts derived from such cells, and more recently in yeast cells. However, an extract derived from wheat germ has been used in many experiments, in parallel with the most widely used extract of rabbit reticulocytes. In addition, in recent years a variety of experiments have been performed with transiently or stably transformed plants or plant cells. It was concluded from these experiments that most translational features are similar in plant and animal cells ([127, 320] for review). Some plant translation factors have been characterized and the genes cloned (see review by Browning, this issue). Some of the data suggesting general similarities between translation in mammalian and in plant cells are discussed below.

Cap-dependent ribosome binding and translation initiation

The vast majority of eukaryotic mRNAs are capped at the 5' end, polyadenylated at the 3' end, and monocistronic, i.e. only one open reading frame (ORF) is translated into a protein per mRNA molecule. Translation of such mRNAs is well explained by the scanning model, which states that 40S ribosomal subunits – associated with eukaryotic initiation factors (eIF) 1A, 3 and the 'ternary complex' of eIF2, GTP and initiator methionine tRNA – bind near the capped 5' end of the mRNA, which in turn is associated with a set of factors of the eIF4 group. The function of the members of the eIF4 group seems to be to prepare the RNA for ribosome binding by removing hindering secondary structures and promoting the linear migration of the bound ribosomes along the RNA in search of a translation start site (scanning). At such a start site, usually an AUG codon, the 60S ribosomal subunit joins on and the resulting 80S subunit begins the synthesis of

the encoded polypeptide [162, 202, 208-210, 238, 239, 278, 279, 331].

In the original scanning model, the 40S ribosomal subunit together with a number of initiation factors was defined as the scanning entity [202]. The precise order of the association of factors and ribosomal subunits with each other and with the mRNA is still disputed, and it is possible that more than one pathway can lead to translation initiation, depending on the prevalent conditions [76, 324]. Particularly, ribosome-independent RNA binding of eIF2 has been observed [26, 188]. Alternative hypotheses with factors of the eIF4 group [314] or with eIF2 [332] as the scanning factor have been proposed. According to these latter models, the 40S subunit binds to the mRNA at a later step (possibly only directly at the AUG start codon). We feel that it is more difficult to explain some of the features of eukaryotic translation with these models than with the 40S ribosome scanning model, particularly some aspects of reinitiation of translation at downstream ORFs (see below). In the following, therefore, we discuss translation mainly in terms of the original scanning model.

Cap-dependence

The direct influence of the cap in plant translation systems has been demonstrated in many *in vitro* translation experiments with wheat germ extracts and by transfection of protoplasts with *in vitro* synthesized RNAs containing or lacking a cap structure [16, 48, 53, 102, 120, 123, 240, 327, 334, 337]. The competitiveness of AMV (for virus acronyms, see Table 1) RNAs for the translation machinery was directly related to cap accessibility [134]. The cap dependence of *in vitro* systems in general depends on the extract used, on the precise reaction conditions [202, 224], on the concentrations of the different initiation factors [102, 324] and on the specific RNAs ([218, 217, 334]; see also the review by Gallie, this issue). Stimulatory effects of the cap on expression in the range of 2- to 100-fold were observed. The wheat germ extract is generally more cap-dependent than the rabbit reticulocyte lysate [224]. *In vivo*, the effect of the cap structure was partially to stabilize the RNA but the main influence was clearly on translation efficiency [123, 124].

The importance of the RNA 5' end for translation was also indicated by the strong inhibitory effect of an inserted stem structure (–125 kJ/mol) at the immediate 5' end of an RNA encoded by a transfected nuclear gene [112, 114].

Table 1. Virus acronyms.

AMV	Alfalfa mosaic	<i>Alfalfa</i>
BMV	Brome mosaic	<i>Bromo</i>
BWYV	Beet western yellows	<i>Luteo</i>
BWYV-ST9	BWYV associated RNA	<i>Satellite</i>
BYDV	Barley yellow dwarf	<i>Luteo</i>
BYDV-RMV	Barley yellow dwarf	<i>Luteo</i>
BYDV-RPV	Barley yellow dwarf	<i>Luteo</i>
BYV	Beet yellows	<i>Clostero</i>
CaMV	Cauliflower mosaic	<i>Caulimo</i>
CarMV	Carnation mottle	<i>Carmo</i>
CCFV	Cardamine chlorotic fleck	<i>Carmo</i>
CERV	Carnation etched ring	<i>Caulimo</i>
CfMV	Cocksfoot mottle	<i>Sobemo</i>
CNV	Cucumber necrosis	<i>Tombus</i>
CPMV	Cowpea mosaic	<i>Como</i>
CRSV	Carnation ringspot	<i>Diantho</i>
CTV	Citrus tristeza	<i>Clostero</i>
CyRSV	Cymbidium ringspot	<i>Tombus</i>
EMV	Eggplant mosaic	<i>Tymo</i>
FMV	Figwort mosaic	<i>Caulimo</i>
KYMV	Kennedya yellow mosaic	<i>Tymo</i>
MCMV	Maize chlorotic mottle	<i>Sobemo</i>
MNSV	Melon necrotic spot	<i>Carmo</i>
OYMV	Osirio yellow mosaic	<i>Tymo</i>
PCV RNA1	Peanut clump	<i>Sobemo</i>
PEMV	Pea enation mosaic	<i>Enamo</i>
PLRV	Potato leafroll	<i>Luteo</i>
PCISV	Peanut chlorotic streak	<i>Caulimo</i>
PPV	Plum pox	<i>Poty</i>
PVM	Potato M	<i>Carla</i>
RCNMV	Red clover necrotic mosaic	<i>Diantho</i>
RDV	Rice dwarf	<i>Phytoreo</i>
RTBV	Rice tungro bacilliform	<i>Badna</i>
RYMV	Rice yellow mottle	<i>Sobemo</i>
SBMV	Southern bean mosaic	<i>Sobemo</i>
SBWMV	Soil-borne wheat mosaic	<i>Sobemo</i>
SDV	Soybean dwarf	<i>Luteo</i>
SoCIM	Soybean chlorotic mottle	<i>Caulimo</i>
STMV	Satellite tobacco mosaic	<i>Satellite</i>
STNV	Satellite tobacco necrosis	<i>Satellite</i>
TBSV	Tomato bushy stunt	<i>Tombus</i>
TCV	Turnip crinkle	<i>Carmo</i>
TEV	Tobacco etch	<i>Poty</i>
TMV	Tobacco mosaic	<i>Tobamo</i>
TNV	Tobacco necrosis	<i>Necro</i>
TRV	Tobacco rattle	<i>Tobra</i>
TuMV	Turnip mosaic	<i>Poty</i>
TYMV	Turnip yellow mosaic	<i>Tymo</i>

Scanning

The ribosome migrates along the RNA from the first point of ribosome association to the translation initiation site. Evidence for such a scanning process is obtained from the of insertion of strong secondary structures or additional initiation sites into the scanned region. The scanning ribosome or its associated factors are apparently unable to melt stem structures of a given stability [14, 199]. The required minimal stability has not been determined for plant cells, but hairpins with a free energy below -180 kJ/mol [16, 112, 114, 204] were sufficient to significantly inhibit translation of downstream ORFs in a variety of plant protoplast and *in vitro* systems. Similarly, the introduction of alternative initiation sites reduces downstream translation because the ribosome ‘wastes’ its initiation capacity at these sites (see below). Both findings show that the ribosome encounters the inhibiting features on the way to the normal initiation site, i.e. it most likely migrates along the RNA. The region upstream of the main initiation site is termed the ‘leader sequence’ or ‘5’ untranslated region’, although the latter designation could be misleading since in some cases such a region might actually contain short ORFs which may be translated (see below).

Only a small number of leader sequences of nuclear plant genes have been systematically compared [186]. The analysis is complicated by the frequent lack of precise RNA data for plant genes. However, it can be concluded so far that the leader sequences of most plant genes (like those of other eukaryotic genes) are shorter than 100 nucleotides, lack strong secondary structures and short ORFs and are thus suited to the ribosome-scanning mechanism. A few exceptions will be discussed below. The leader sequences of many RNA viruses seem to have primary or secondary structures that allow a particularly efficient translation, often even in the absence of a cap structure. These features are discussed in detail by Gallie (this volume). In general, longer leaders (e.g. 80 nucleotides) result in higher translation rates than shorter leaders (e.g. 10 nucleotides). The exact difference depends on the assay system and salt conditions [201]; in transfected plant protoplasts an approximately twofold effect was reported [126], while the effects in the reticulocyte lysate can be greater [206]. However, mRNAs with leader sequences less than 10 nucleotides long can still be efficiently translated in plant cells (G. Chen *et al.*, unpublished observation) and are actually found

in some coat protein mRNAs of RNA viruses (e.g. BMV).

Translation-initiation site selection

Upon initiation, the ternary complex dissociates from the ribosome as eIF2-GDP. Since, according to the scanning model, the ternary complex is delivered to the initiation codon together with the ribosome, ribosomes can initiate translation only once unless a new ternary complex can be recruited. Due to the scanning process, this one-and-only initiation site is the one located closest to the 5' end of the RNA. Translation usually begins at an AUG codon, and the efficiency of AUG codon recognition is modulated by the sequence context of this codon [198]. Sequence compilation of plant translation initiation regions has suggested that the most frequent (and therefore regarded as optimal) AUG context for plant genes is similar to that of mammalian genes (AACAAATGGCG [55, 186, 227]). The most crucial positions in both cases are a purine at position -3 and a guanine at position +4 (where the A of the AUG is +1). For mammalian cells, an influence of positions +5 and +6 has been recently documented [32, 144]; other positions seem to be less important. Start codons that deviate from the optimal context at one or more of the crucial positions may be recognized less efficiently and allow the passage of ribosomes to further downstream start codons (leaky scanning). The influence of sequence context on initiation in plant cells has been directly verified by mutagenesis studies *in vitro*, in plant protoplasts, and in transgenic plant cells [6, 77, 87, 146, 203, 220, 253, 328]. Differences in initiation efficiency between codons in optimal or suboptimal contexts strongly depend on the conditions of the assay system [87, 203]. While some workers concluded that the context of the initiation codon in plants is of minor importance [227], others have shown that reduction of the Mg²⁺ concentration from 3 mM to below 2 mM produces significant codon context discrimination, similar to that in mammalian systems [86, 203]. However, it is unclear whether these optimal *in vitro* conditions also apply to translation *in vivo*. The (sub-optimal) codon context of soybean lipoxygenase ORFs (AAAG.ATG.TTT) was found to give 10 times higher β -glucuronidase (GUS) expression rates than the (also unfavorable) context in the standard GUS expression plasmid pBI 211 [192]. In transgenic plants, translation initiation patterns from two consecutive, in-frame AUG codons were found to be drastically different in

different parts of the plant (and at different developmental stages) [161].

The efficiency of start-codon recognition can be influenced by features that lead to a prolonged pause of the scanning ribosome at the position of the codon. A secondary structure element located 14 nt downstream of the start codon was found to increase initiation efficiency at weak AUG and at non-AUG codons [205]. A similar pause results from a downstream initiation event and can cause more efficient recognition of a properly spaced upstream AUG [87]. In contrast, initiation at a downstream codon may be negatively influenced by overlapping translation from an upstream start site, possibly because of interference by the translating 80S ribosomes [211].

Translation elongation

Decoding of the RNA occurs at the aminoacyl tRNA site (A site) of the ribosome in 3-base steps by an amino-acylated tRNA with appropriate anticodon. Peptide elongation occurs by transfer of the nascent peptide from the tRNA of the previous decoding step, which is located at the peptidyl tRNA site (P site), to the aminoacyl tRNA at the A site. The mRNA(codon)-tRNA-peptide complex is then translocated to the P site and the A site becomes free for interaction with the next tRNA. A third site (exit or E site) on the ribosome interacts with the unloaded tRNA before release. The binding of a tRNA to its codon differs in the A and P sites [272] and interactions between the tRNAs at the different ribosomal sites influence the kinetics of decoding [222, 312]. Translation elongation is promoted by eukaryotic elongation factors eEF1 and eEF2 and requires the hydrolysis of GTP [281]. Unlike the scanning process, ribosome translocation during peptide synthesis is relatively insensitive to inhibition by RNA secondary structure. Even basepairing between long sense and anti-sense RNA stretches can be disrupted, provided it begins more than about 20 nt downstream of the AUG codon and, therefore, does not interfere with the formation of the 80S ribosome [185, 221, 300]. Structural elements may nevertheless cause transient pausing of translating ribosomes (see below). The speed of translation elongation can vary between less than 2 to about 10 amino acids per second per translating ribosome in eukaryotic cells, depending on the cellular conditions and the mRNA ([289] for review). While initiation is generally the rate-limiting step in translation, elongation can become limiting with some particular ORFs [62, 94] or under certain physiologic-

al conditions [289]; for example, elongation rates are reduced at the slightly acidic pH that is induced by oxygen deprivation [354]. For plants, regulation at the level of elongation has been proposed for two oat seed proteins [37] and in response to heat-shock [10], oxygen deprivation [99, 348, 354], wounding [248], and light [28, 305, 310].

The nascent peptide is transported through a ribosome tunnel to the surface [71, 364], where it can interact with signal recognition particles or other cytoplasmic factors [359]. In bacteria, slowing down of elongation can be caused by the nascent peptide [145, 226], and the penultimate amino acid in particular may influence the termination step [245, 249]. These effects are probably caused by interactions between the peptide and translation factors, rRNAs or the tunnel through which it is transported to the surface of the ribosome [154], and it is likely that similar mechanisms exist in eukaryotes. Ribosomal pause sites not ascribed to secondary structure elements have been detected for a number of genes [193, 194, 263, 362]. It has been proposed that codons that are recognized by rare tRNAs ('rare codons' [47]) interfere with efficient translation [371] and could be a regulatory feature of gene expression [66, 291]. The speed of decoding could also influence the folding of the nascent peptide chain [213], and possibly the targeting [263]. In microorganisms, rare codons tend to be avoided in highly expressed genes and adjustment of the codon bias can result in increased expression of a gene [78, 315]. In yeast [166] and *Escherichia coli* [59, 135], a number of rare codons near the initiation site is required to substantially reduce expression of the respective ORF.

It is likely that all these mechanisms also apply to translation in plant cells. A compilative analysis of protein-coding sequences revealed that the codon usage in plants differs from other organisms, and differences can also be discerned between plant families [49, 252]. In plants, particularly in monocots, highly expressed genes show no special preference for frequent codons [49, 252]. Nevertheless, bacterial genes have been modified to comply with the plant codon usage and were indeed expressed to a much higher level [107, 212, 267]. During this mutagenesis, however, signals leading to potential aberrant RNA processing were also altered, and it is still not clear which of the features of the new RNA sequence are responsible for the elevated expression.

Translation termination

For the stop codon (UAA, UAG, UGA) usually no complementary tRNAs are available and release factors associate with the ribosome; this causes release of the polypeptide chain and termination of translation [45, 69, 338, 344, 345]. In all eukaryotes studied so far, the three stop codons are recognized by only one release factor, eRF1 [106, 372] or SUP45 in yeast [317]. Recognition or termination activity is stimulated by a second protein factor, eRF3 [372] or SUP35 in yeast ([317, 318] for review). The immediate sequence context of stop codons can modulate recognition efficiency. Hierarchies of termination efficiency have been established for *E. coli* [271], mammals [236], and yeast [33], and sequence comparisons suggest the existence of similar context effects in other eukaryotes, including plants [9, 42, 43, 55, 344]. The fate of the ribosome after translation termination remains unclear. However, at least some ribosomal subunits must resume scanning after translation termination since in some cases ribosomes are able to reinitiate translation at other downstream start sites. It has been proposed that the reinitiation capacity is modulated by sequences surrounding the stop codons [142, 242], but it is not known whether this occurs at the level of ribosome-bound factors or at the level of ribosome-RNA association.

The exceptions

Translation of simple, monocistronic RNAs is easily explained by the scanning model. The translation of exceptional RNAs with unconventional initiation sites or encoding more than one protein or peptide, discussed in the following sections, is not fundamentally different but requires a few modifications or additions to the original model. Most of these RNAs are derived from plant viruses [168, 243, 285].

Variations of initiation site selection

Non-AUG start codons

Translation in mammalian and insect cells can commence at codons which differ from AUG in one position. In artificial constructs, most of the possible AUG derivatives are active to some extent [203, 237, 262]. In natural mRNAs, CUG is mainly found as an alternative start codon [36, 54, 151, 273, 342, 343], but indica-

tions of the activity of AUC and ACG have also been presented [19, 72]. In *Saccharomyces cerevisiae*, non-AUG codons are recognized very inefficiently [65]. Non-AUG recognition can be enforced by mutations in eIF2 β [89] and eIF2 γ [90]. For plants, the activity of such codons was analyzed with artificial constructs in wheat germ extracts [203] and in transfected protoplasts [138]. In both systems, initiation at non-AUG codons was more context dependent than that at AUG codons, and in the wheat germ extract recognition was much higher at high Mg²⁺ concentrations [203]. For protoplasts, a hierarchy of activity was found of CUG (30% efficiency of an AUG codon), GUG, ACG (15%), UUG, AUA, AUC, AUU (2 to 5%), and AAG, AGG (<0.1%) [138].

It has been inferred that non-AUG initiation occurs in a mutated version of a CaMV RNA at the start of the *pol* gene [297]. This example is important in that it shows that mutation of the start codon of an ORF does not necessarily abolish ORF expression completely.

An AUU codon opens the first ORF of rice tungro bacilliform virus (RTBV). Translation efficiency at this codon is low in RNAs where the position is reached in a normal scanning mechanism but reaches about 10% of AUG efficiency at its natural position downstream of the long RTBV leader sequence [116]. This leader sequence contains a number of features that inhibit scanning and most likely a specific mechanism is required for ribosomes to reach the region downstream of the leader (see below). This mechanism may also lead to enhanced recognition of the AUU codon.

No other plant genes with translation initiation exclusively at non-AUG codons have been described. In mammals, most ORFs with a non-AUG initiation site also have a normal AUG initiation site further downstream. Additional non-AUG initiation is found mainly for ORFs encoding regulatory proteins that are located downstream of a long, structured leader sequence. The protein variants with N-terminal extensions may have regulatory effects different to the 'normal' one [2, 46] and initiation at the additional sites can be regulated by the cellular conditions ([151, 152] for review). Similar translation events may occur in plants, but these rare and probably important events have not yet been detected because few plant genes and their protein products have been analyzed to the required extent. In soil-borne wheat mosaic virus (SBWMV), an N-terminal extension variant of a 29 kDa protein with unknown function may be produced by initiation at an as yet unidentified, non-AUG codon upstream of the first AUG codon on RNA 2 [303].

80S ribosome formation at an AUU codon in the leader sequence of TMV RNA (Ω) has been reported [101, 173, 340]. The codon is in frame with the TMV 126 kDa protein but it is not known whether it serves as an initiation site for a variant of this protein. An ORF fused to the Ω leader was found to be translated also from an upstream AUU codon with high efficiency [323]. The 5' leader sequence of AMV RNA 3 even binds two 80S ribosomes, presumably also at AUU codons [268]; one 80S ribosome also binds to an as yet unassigned position in the leaders of BMV RNA3 [5] and TYMV RNA [101]. The relevance of these binding events for translation of the respective RNAs remains unclear. Disome or trisome formation is not involved in the translation stimulating effect of these leader sequences [126, 309].

Non-AUG initiation can occur at inconspicuous positions of an mRNA: in the leader sequence of the CaMV 35S RNA, efficient initiation at a CUG codon that leads to translation of a short ORF has been detected (discussed by Gordon *et al.* [138]). Again, it is not known whether this translation event has any functional importance, but the example shows that caution is required when the possible translation events on one RNA are only deduced from sequence data.

RNAs with multiple translation initiation sites

If the translation start site for an ORF is not the most 5'-proximal on a given RNA, three different possibilities exist for the route by which a translation-competent ribosomal subunit may reach this codon (Fig. 1).

1. Most simply, but least compatible with the scanning model, ribosomes avoid upstream initiation sites by jumping or by directly binding to a downstream site.
2. In line with the scanning model, the upstream codon may be avoided by a fraction of ribosomes due to effects of the sequence context (leaky scanning). A special case for leaky scanning are start codons that are so close to the RNA 5' end that the decoding site of the 43S subunit is already downstream when the ribosome is bound to the 5' end [206, 298].
3. In cases where the upstream ORF terminates before the downstream initiation site, ribosomes may continue scanning and reinitiate translation; reinitiation requires the recruitment of initiation factors by the scanning ribosomes.

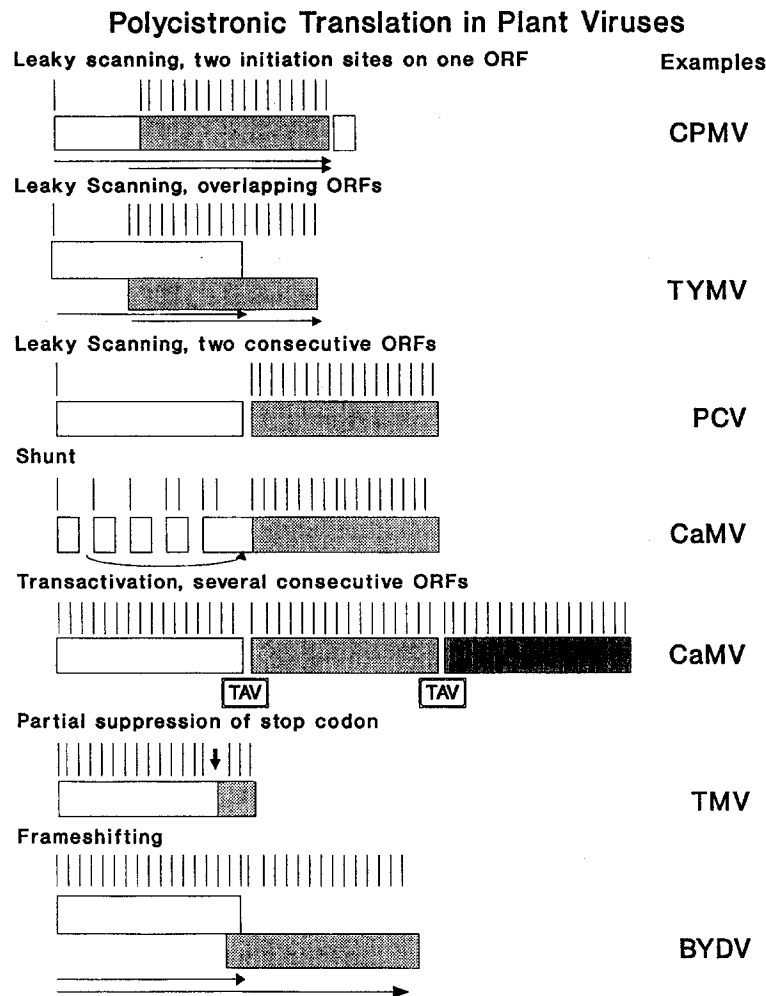


Figure 1. Strategies of polycistronic in plant viruses. The arrangement of the ORFs is shown schematically. The series of line marks above the ORFs represent the AUG codons. The horizontal arrows represent the movements of translating ribosomes, the bend arrow the movement of the shunting ribosome or complex. The vertical arrow points to the site of the partially suppressed stop codon. Examples for viruses using the polycistronic translation strategies are given at the right.

Examples of all these mechanisms are known for specific mammalian RNAs [207] and at least some have been described for RNAs translated in plants.

RNAs with multiple initiation sites can be grouped in three classes (Fig. 1):

1. RNAs that contain one or more short ORFs (sORFs) in their leader region; the sORF may terminate before or overlap the main ORF;
2. RNAs that contain multiple, overlapping long ORFs;
3. RNAs that contain multiple, non-overlapping long ORFs.

The distinction between a 'coding, long' ORF and a sORF is somewhat arbitrary. Usually a sORF is shorter

than 50 codons and the putative translation product is not known to have any function besides possibly regulating the translation efficiency in *cis*.

Short ORFs in the 5' leader sequence

Systematic comparison of the 5' leader sequences of 79 plant genes showed that 6 of these contain upstream AUG(s) [186]. Our own, unsystematic inspection of plant gene sequences also showed that about 10% contain one or more AUG codons in the 5' leader sequence. In many cases, it remains to be seen which of these AUG codons are present in functional mRNAs; some may be removed by splicing. Unspliced RNAs are not

necessarily degraded in plant cells [136] and may be represented in cDNA preparations. In other cases, multiple transcription start sites may lead to the production of mRNAs with different 5' leader sequences. The effect of upstream sORFs on translation has been studied for a few nuclear-encoded mRNAs: The mRNA for the maize *Lc* protein contains a 38 codon long sORF (with a total of three AUG codons) in its 256 nucleotide long leader region, which ends 62 nucleotides upstream of the *Lc* initiation codon [73]. As expected from the scanning model, the presence of this sORF causes a reduction in *Lc* translation. In an assay that used the transcription-transactivating activity of the *Lc* protein for quantification of *Lc* translation, a 30-fold repression was observed in biolistically transfected maize aleurone cells. The repressing effect depended greatly on the intactness of the sORF sequence; all point mutations alleviated the effect at least partially. The mutations also included some which led to conservative amino acid exchanges or were even silent [73]. Examples in which the coding sequence of an sORF is important for its repressing effect are documented for yeast [242, 358] and mammalian cells ([50, 80, 133] for review) but the stringent sequence requirements observed for the *Lc* leader sORF are unique. It is noteworthy, that the mutated versions of the sORF apparently had almost no inhibitory effect on downstream translation although they still contained AUG codons and should therefore be translated.

The mRNA leader of the maize *opaque-2* gene contains three, partially overlapping sORFs which inhibit translation of the downstream *opaque-2* ORF about fivefold in tobacco protoplasts [225]. Individual sORFs showed a similar inhibitory effect. When the leader sequence was modified such that either the first or second sORF was elongated to overlap the downstream, long ORF, inhibition was increased. This is taken as evidence that at least the first two sORF initiation sites are actually used as efficient translation start sites and that the normally observed initiation at the *opaque-2* ORF AUG is a reinitiation event [225]. As stated above, it is possible that even a poorly translated overlapping ORF may interfere with downstream initiation [211] and it cannot be excluded that the *opaque-2* ORF is normally translated by leaky scanning, which is inactivated in the overlapping ORF configuration.

The leader sequences of several plasma membrane proton ATPases from *Nicotiana plumbaginifolia*, tomato and *Arabidopsis thaliana* also have sORFs. The sORF in the *pma1* RNA is 10 codons long, contains two AUG codons in suboptimal sequence context

and ends about 60 nucleotides upstream of the coding ORF. Removal of any single AUG codon had no effect on translation but removal of both increased translation about twofold in wheat germ extracts and in protoplasts [241].

The leader sequences of several *Arabidopsis thaliana* homeobox genes contain sORFs. For the homeobox gene 1 (*ATH1*), the leader is longer than 600 nucleotides. The 5' halves of the probably two different mRNA versions have not yet been characterized but the 3' half contains a number of sORFs and inhibits translation in an *in vitro* system about fivefold [276]. It is unknown whether the leader is involved in the complex regulation of *ATH1* activity.

An sORF in the leader of the soybean aminoalcohol-phosphotransferase 1 (*AAPT1*) inhibited translation of the downstream ORF in yeast and thereby interfered with complementation of a yeast mutant by this plant gene [84]. Again, it is unknown whether the sORF regulates *AAPT1* expression in plants.

Negative effects of sORFs on downstream translation also have been observed in artificial constructs made for plant transformation [31, 275, 284]. The effects of several features of an upstream sORF were tested in constructs in which a reporter ORF was preceded by an artificially designed sORF. The length of the sORF was modulated by sequence multiplication and the number of potential start codons was varied. The inhibitory effect increased with the length of the sORF. Even the shortest possible sORF, consisting only of an AUG codon, reduced downstream translation by about a factor of two. Intermediate length sORFs (around 30 codons) reduced fivefold and sORFs longer than 100 codons completely abolished downstream translation [113]. In plant protoplasts, sORFs with additional internal AUG codons were not more inhibitory than those without, probably because the first start codon was in an optimal sequence context and thus all approaching ribosomes initiated there. If the sORF overlapped the downstream ORF, translation was completely precluded. A limited number of sORF sequence variants in the coding sequence or around the stop codon were also tested, but no further features influencing the inhibitory potential of the sORF could be discerned [113]. From all these results it is clear, that sORFs can be inhibitory because they are translated and thus reduce translation initiation at the downstream start codon. However, initiation at an sORF does not completely preclude translation reinitiation at a further downstream ORF by the same ribosome [105, 113, 275]. This process should require the recruitment of a

new set of initiation factors. In the artificial constructs described by Fütterer and Hohn [113], the reinitiation efficiency was dependent on the length of the sORF and reinitiation was observed despite an intercistronic distance of only 16 nucleotides.

The effect of sORFs on translation of a further downstream ORF has also been studied in mammalian and yeast systems. A similar length dependence of the inhibitory effect of the sORF was observed in mammalian cells [228]. Besides, on the sORF's coding sequence (see above), the efficiency of downstream translation was also dependent on sequences downstream of the sORF's termination codon [142] and on the distance between sORF and downstream ORF. Distances greater than 50 [200] or 200 [1] nucleotides were required and in the yeast system, downstream translation was strongly influenced by the physiological state of the cells ([1, 165, 260, 358] for reviews). Tissue-specific effects of sORFs on downstream translation have also been observed in mammalian cells [375]. It is likely that similar effects also exist in plants. So far the only safe fact seems to be that an sORF-containing leader always causes lower expression than an sORF-free leader. In particular, for long leader sequences with several sORFs, the multitude of possible activities of ribosomes, such as translation, leaky scanning, stalling, translation-termination-dependent dissociation from the RNA, non-productive scanning (due to the lack of initiation factors), sequence- or distance-dependent reinitiation etc., makes a precise prediction of the degree of inhibition impossible.

Up to 14 AUG codons are found in the leader sequences of the pregenomic RNAs of the plant pararetroviruses, and the leaders of the CaMV 35S RNA [17, 109] and the RTBV 35S RNA [58] are indeed inhibitory to downstream translation. For CaMV, inhibition was up to 100-fold in some protoplast systems derived from non-host plants [17, 109]. In other protoplast systems, including some from virus host plants, the inhibition was much less severe (around two- to six-fold; [109]). These leader sequences can also form extensive secondary structures [108, 147, 158]. It is possible that most of the translation inhibitory effect of the leader was caused by secondary structure rather than by the sORFs, as was shown for the *c-sis* proto-oncogene RNA with its long, sORF-containing leader [170]. It is, however, likely in CaMV that some of the sORFs inhibit downstream translation since they are inhibitory when located in the supposedly unstructured leaders of truncated mRNAs. The first three CaMV sORFs are very short (2–5 codons) and their

start codons are in suboptimal sequence context. Consequently, they are only slightly inhibitory for translation of a reporter gene fused to sORFs located further downstream [108, 114]. The longer sORFs in the 3' half of the leader, at least one of which has a start codon in optimal sequence context, are more inhibitory [111]. Translation of a reporter ORF positioned in the center of the leader follows the scanning model. Either enough ribosomes avoid initiation at the upstream sORFs because of the unfavorable sequence context or they regain initiation capacity after sORF translation [112, 114]. The first longer ORF downstream of the CaMV 35S RNA leader is thought to be translated by modified scanning which was termed ribosome shunt [114]. A similar process might be active on the RTBV 35S RNA [116]. In the shunt, initially scanning ribosomes are transferred directly from a donor to an acceptor site without linear scanning of the intervening region. The ribosome migration on the 35S RNA leader has been studied by insertion of strong stem-loop structures and additional ORFs at various sites. (It is noteworthy that in all cases where no ribosome shunt was involved, these additional elements produced exactly the effect that would have been predicted by the scanning model. Therefore, these experiments are also another confirmation of the general features of the scanning model in plant cells.) The shunt could also be detected *in trans*, albeit at low efficiency [114] and was independent of viral proteins. Since shunt efficiency showed a certain cell-type dependence, involvement of cellular factors is likely [109]. Although mutagenesis analyses have defined regions in the leader sequence of the CaMV and RTBV 35S RNAs that are important for the shunt process [58, 111, 114, 116], the structural features that allow a shunt are only poorly defined so far. It is assumed that long-range RNA interactions are involved, probably with the assistance of associated proteins. Computer programs predicted a stable stem-loop structure for almost the complete CaMV and RTBV leader sequences [108, 158], and for CaMV such a structure was also predicted by an analysis of folding parameters of the growing RNA molecule [147]. Direct analysis of the *in vitro* structure of the leader confirms the presence of the predicted structure or a similar one (M. Hemmings-Mieszczyk, G. Steger, and T. Hohn, unpublished observations). In an alternative folding of the CaMV 35S RNA leader sequence, the presence of pseudo-knots in the acceptor as well as in the donor regions was noted (K. Gordon, pers. comm.). Which of these structures, if any, is important for the ribosome shunt has still to

be determined. The location of the ribosome acceptor region was defined for CaMV and for RTBV with some precision. In both cases, it lies immediately downstream of a CT-rich region and for RTBV it involves the AUU start codon of the first ORF [114, 116]. The efficiency of recognition of this AUU start codon is increased above that of the same codon reached by scanning, possibly because a shunted ribosome has more time for recognition before scanning is resumed [116]. Recently, translation of CaMV 35S RNA leader constructs in wheat germ extracts produced results that suggest that a ribosome shunt can also occur in this heterologous *in vitro* system (W. Schmid-Puchta, D. Dominguez and T. Hohn, unpublished observations).

Ribosome shunt-like mechanisms have been described for translation of Sendai virus RNA [72], the adenovirus tripartite leader (A. Yueh and R.J. Schneider, pers. comm.) and for a papova-virus (G. Hobom, pers. comm.).

RNAs with more than one coding ORF

RNAs that encode more than one protein in consecutive or overlapping ORFs have not been described for plants; however, a number of plant viruses use such RNAs (Fig. 1) and apply one or several unusual mechanism(s) for their translation (Fig. 2).

In the most easily explainable cases, translation initiation at two start sites is regulated by the rules of leaky scanning. This requires that the first AUG is in an unfavorable sequence context. The second initiation site can be in the same or in a different reading phase, giving rise to an N-terminal deletion variant of the first protein, or a completely different protein, respectively. The degree of leakiness of an AUG codon can be high; a potential start codon in the leader of PPV was found to be recognized very inefficiently [280] while for two ORFs of the luteoviruses PLRV and BYDV-PAV ratios between 100:1 and 1:7 for translation of two overlapping ORFs have been described [86, 87, 322]. This mainly depends on the sequence context [87] and on the conditions of the translation system (cation concentration [89]). A protoplast system was more discriminating (100-fold difference) between an optimal and a suboptimal start codon than the reticulocyte lysate [87]. In those cases where leaky scanning has been observed or postulated, the first start codon is usually in an unfavorable sequence context (Table 2).

As described above, the ratio of expression from two overlapping ORFs depends not only on the direct

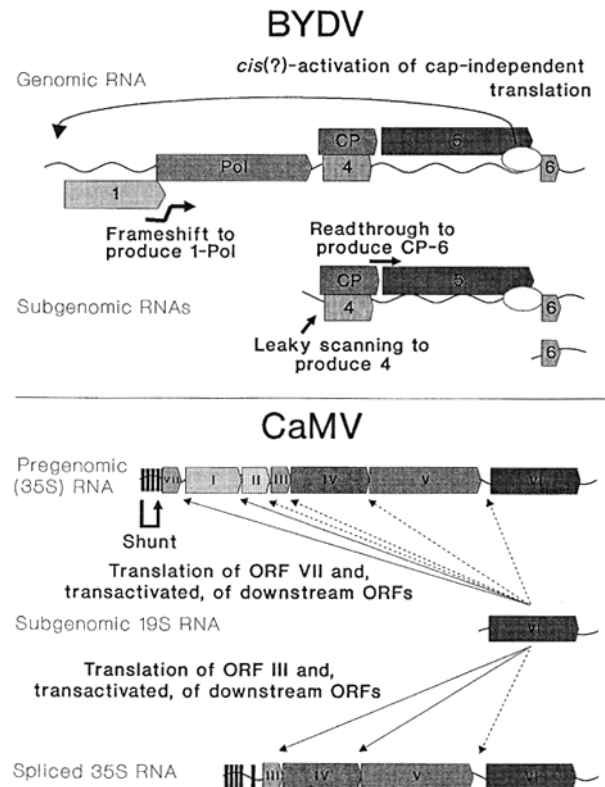


Figure 2. Expression strategies of luteo- and caulimoviruses exemplified by BYDV and CaMV, respectively. The genomic RNA and the subgenomic mRNAs are shown including their ORFs. *BYDV*: ORF 1, CP and ORF 6 are translated as first ORFs from the three RNAs. Pol is translated as ORF1-Pol fusion protein upon a frameshift linking the two ORFs from the full length RNA. ORF 4, which overlaps with CP is translated upon leaky scanning and ORF 5 is translated as CP-ORF 5 fusion protein by readthrough of an amber stop codon from the longer subgenomic RNA. A site close to the 3' end of the two larger RNAs promotes translation *in cis*. *CaMV*: Three mRNAs were identified, the pregenomic 35S RNA, the subgenomic 19S RNA and a spliced version of the 35S RNA. The first ORF (ORF VII) of the 35S RNA is translated after a shunt mechanism. Whether translation of the first ORF (ORF III) from the spliced RNA also requires shunting or another mechanism (leaky scanning, reinitiation) is not known. Translation of the second and probably consecutive ORFs from unspliced and spliced 35S RNA occurs upon transactivation involving the translation product of ORF VI (transactivator) derived from the 19S RNA. The transactivator is the only larger CaMV protein obtained by classical translation.

sequence context of the start codons but may also be influenced by structural features of the RNA. In mammalian cells, mutual influences of overlapping ORFs during the elongation phase of translation have been described, probably caused by the different speed of decoding of the two overlapping reading phases [94]. A similar effect may account for observations made with start codon mutations of the overlapping TYMV ORFs

Table 2. Leaky start codons of plant viral ORFs. The immediate sequence context of start codons known or supposed to be leaky is presented. Nucleotides that conform to the general eukaryotic start codon consensus sequence (A/G)NNAUGG are highlighted in bold. The distance to the next downstream AUG codon and the ORFs in whose translation the start codons are involved are listed. References to viruses not further mentioned in this review can be found in the compilation by Miller *et al.* [243].

	ORFs	Leaky AUG codon	Distance to second AUG (nts)	Ref.
Overlapping ORFs				
<i>Luteovirus</i>				
PLRV-S,A,C	ORF0/ORF1	CAU. AUG .AU	128	243
BWYV		UUG. AUG .CA	137	
RPV	"	CGC. AUG .UU	146	
BYDV-PAV, MAV, RMV	ORF3/ORF4	UGA. AUG .AA	14–41	
BYDV-RPV	"	UUA. AUG .AG	20	
SDV	"	AGU. AUG .GU	11	
BWYV	"	UUA. AUG .AA	26	
PLRV	"	UUA. AUG .AG	20	
<i>Enamovirus</i>				
PEMV RNA1	ORF0/ORF1	UUU. AUG .CA	77	
PEMV RNA2	25K/27K	UAU. AUG .AC	11	
<i>Carmovirus</i>				
MCMV	p31.6/p50	UUC. AUG .CC	14	
<i>Tymovirus</i>				
TYMV	replicase	CAA. AUG .AG	2	247
EMV	"	UCA. AUG .CC	2	
KYMV	"	CUG. AUG .UC	2	
OYMV	"	UUC. AUG .UC	2	
<i>Tombusvirus</i>				
CNV, TBSV, CyRSV	21K/20K	UUC. AUG .GA	27	283
<i>Phytoreovirus</i>				
RDV (S12 RNA)	p34/p10.5	AUA. AUG .UU	267	
<i>Satellite virus</i>				
STMV	p6.8/p17.5	UUU. AUG .CU	104	244
Two initiation sites in one ORF				
<i>Potyvirus</i>				
PPV	Polyprotein	UUU. AUG .CA	106	280
<i>Comovirus</i>				
CPMV M-RNA	Polyprotein	ACA. AUG .UU	349	350
<i>Phytoreovirus</i>				
RDV (S12 RNA)	10.5K ORF	UUA. AUG .CU	19	244
<i>Badnavirus</i>				
RTBV (spliced RNA)	ORF IV	UCA. AUG .GC	71	115
Independent ORFs				
<i>Furovirus</i>				
PCV RNA2	coat protein/p39	CUU. AUG .UC	617	164
<i>Badnavirus</i>				
RTBV	ORFs I and II	AAU. AUU .GA	562	158
	ORFs II and III	UAC. AUG .AG	325	

for a 69 kDa and a 206 kDa protein, where it was found that elimination of the first AUG had no positive effect on translation from the second one, while elimination of the second AUG increased translation from the first one [355]. This suggests that translation (initiation or elongation) of the downstream ORF is also rate limiting for the overlapping upstream ORF. Initiation efficiency at the upstream BYDV coat protein (CP) ORF was positively influenced by efficient initiation of the 17 kDa ORF starting 40 nucleotides downstream. Probably the pause resulting from initiation at the downstream AUG results in ribosome stacking and provides more time for recognition of the upstream AUG [87].

Leaky scanning is at least partially responsible for translation of the 95 kDa protein of CPMV M RNA, which initiates about 350 nucleotides downstream of the 105 kDa protein; no AUG codon in any of the three reading phases is found in this intervening region [350]. Translation of RNAs of the rice dwarf phytoeovirus [321], the satellite tobacco mosaic virus [244] and luteo-, tymo- and tombus viruses also probably involves leaky scanning ([243] for review).

In at least two cases, leaky scanning is the mechanism of translation of subsequent, non-overlapping ORFs. An ORF on PCV RNA 2 is located downstream of the 620 nucleotides long coat protein ORF but is accessible to scanning ribosomes because this long upstream region is devoid of AUG codons apart from the one opening the CP ORF. Leaky scanning was deduced from the negative effect on downstream translation of the insertion of stem structures or of additional AUG codons into the upstream region [164]. A similar, even more extreme case is found in RTBV, where the 900 nucleotides encoding ORFs I and II upstream of ORF III contain only one AUG codon that opens ORF II and has an unfavorable sequence context. A similarly peculiar bias is also observed in the genomes of the other badnaviruses. Improvement of the efficiency of RTBV ORF I translation by mutating its AUU initiation codon to AUG drastically reduced ORF II and III expression, as expected for a leaky scanning mechanism (unpublished observations).

If the upstream ORF is opened by an efficiently recognized start codon and/or if no particular bias against the presence of additional AUG codons in any of the three reading phases exists, a following ORF will normally not be translated because leaky scanning is impossible and reinitiation of translation is inefficient. In *in vitro* systems, translation efficiencies for downstream ORFs of 1–20% have been found [137], but in plants or protoplasts downstream ORFs are expressed

with considerably lower efficiency. This has generally been experienced with constructs designed to express a marker ORF downstream of another ORF in transgenic plants [8, 174, 374]. An up to 1500-fold reduction of expression efficiency of the downstream ORF was observed [8]; however, even low expression efficiencies can be sufficient to cause phenotypes, such as antibiotic resistance [8, 174] or GUS staining [374]. In one exceptional case, the introduction of a complete bacterial arylsulfatase ORF upstream of a GUS ORF seemed not to influence GUS translation in transiently or stably transformed plant cells [63]. However, in the respective dicistronic constructs, GUS translation begins at an in-frame AUG accidentally present considerably upstream of the AUG that is used in the monocistronic reference construct, and leads to different GUS proteins with possibly different enzyme stabilities or activities. In transient expression experiments in a variety of plant protoplast systems, expression of a downstream ORF was at or below the detection limit [34, 112, 139, 294]. In *Orychophragmus violaceus* protoplasts, detectable expression of a CAT ORF downstream of a GUS ORF was only observed when the intercistronic distance was increased to more than 300 nucleotides [112].

Viral transactivation of polycistronic translation

The pregenomic RNA of the caulimoviruses and probably also a number of internally spliced derivatives of these RNAs serve as polycistronic mRNAs for a number of viral proteins [34, 88, 110, 139, 196, 294]. The respective ORFs closely follow each other without long intercistronic distances, are often opened by efficiently recognized start codons, and usually also contain internal AUG codons. In plant protoplasts and in transgenic plants, most of the downstream ORFs are indeed not or only poorly expressed unless the virus encoded transactivator (TAV) is present (Fig. 2). Transactivation activity has been demonstrated for the ORFs VI of CaMV [34, 83, 112] and FMV [139, 294, 295] and it likely resides also in the corresponding ORFs of CERV, SoCMV, and PCISV [83]. The TAV protein specifically enhances the translation of a downstream ORF. Expression was obtained from totally artificial, polycistronic constructs as long as the ORF organization allowed a reinitiation mechanism, i.e. when long ORF overlaps were avoided [112, 113]. In the artificial polycistronic RNAs, transactivation was particularly efficient when the first ORF was around 30 codons long; shorter and longer ORFs were less effect-

ive [113]. Transactivation was observed for several ORFs following such a short ORF. The polar effects of the insertion of stem-loop structures into polycistronic mRNAs and the specificity for non-overlapping ORFs suggest that transactivation causes enhanced reinitiation of translation. The dependence of transactivation efficiency on first ORF length suggests, that the transactivator directly or indirectly acts on the translating (or terminating) ribosome [112, 113]. The optimal length of the first ORF of 30 codons may be significant since a 30 amino acid nascent peptide is just long enough to emerge from the translating ribosome [231, 359]. It appears possible that at this stage of translation a structural change of the ribosome occurs which leads to loss of a residual reinitiation capacity and also to a loss of transactivation responsiveness. The absence of a requirement for *cis*-active sequences does not necessarily implicate the lack of such sequences in the CaMV genome. The presence of the CaMV ORF VII supported transactivation [34] although an artificial ORF of similar length did not [113]. The expression constructs also contained the CaMV polyadenylation signal, which contributes the terminal 200 nucleotides of the transcripts. Studies with FMV suggested that *cis*-active sequences are required for transactivation in this case. These sequences are located at the end of the FMV 35S RNA leader sequence [140] and in the ORF VI coding region, which was effective as a 3'-untranslated region in the respective plasmid constructs [295]. For the former, however, no distinction was made between sequences that allow ribosome access to the region downstream of the leader (a FMV ribosome shunt) and sequences that are specifically involved in transactivation, nor was the precise configuration of the sORFs with respect to the reporter ORF discussed. In CaMV, the TAV also stimulates translation directly downstream of the leader [114]. This increased expression was dependent on the shunt process and was apparently not caused by the action of the TAV on ribosomes that migrated through the leader sequence. In the case of the stimulating sequences in the 3'-'untranslated' region, it remains to be seen whether the effects are due to stimulation of translation or to RNA stabilization by TAV-induced translation of ORFs in this region. Strong reductions of mRNA levels have been reported for constructs that contained inefficiently translated 3' ORFs [8, 174] and are in general often observed for RNAs with premature stop codons and, therefore, long untranslated 3' regions in plants [347] and other eukaryotes ([232, 259] for review).

The transactivating function could be localized to the central third of the TAV protein [83]. To achieve normal levels of transactivation with a peptide comprising only this portion (mini-TAV), 100-fold more DNA encoding the TAV polypeptide had to be transfected. It is not known whether this lower efficiency is due to a defect in transactivation itself or to problems with protein folding and stability. The mini-TAV was only active in *Nicotiana plumbaginifolia* protoplasts, while the full-length TAV was active in a number of dicot plant protoplasts and also in maize protoplasts [34, 139]. TAV was found to be associated with polysomes and, in an overlay binding assay, also with an 18 kDa ribosomal or ribosome-associated protein from plants and also from yeast (A. Himmelbach, Y. Chapdelaine and T. Hohn, unpublished observations). An RNA-binding activity of TAV residing outside the minimal transactivating region may enhance activity by increasing TAV concentration near the RNA [83]. Crossing of transgenic *Arabidopsis* lines containing either the transactivator or a dicistronic GUS reporter construct revealed that transactivation can also be obtained in transgenic plants [374]. No tissue or development specificity was observed. *Arabidopsis* and tobacco plants expressing the transactivator are not easy to obtain and show abnormal phenotypes [18, 325, 374]. At present it is unclear whether these phenotypes are caused by transactivation or some other activity of the protein. Recently, TAV-dependent transactivation of polycistronic translation was also described for *Saccharomyces cerevisiae* [299]. This latter finding opens up many possibilities for the study of the mechanism and for the characterization of the cellular partners involved.

Leader sequences and cap-independent, internal ribosome entry

Downstream ORFs on a polycistronic mRNA could in principle also be translated in a scanning-independent mechanism by ribosomes that enter the RNA at an internal position (internal ribosome entry site, IRES) as was originally shown for picornaviruses ([3, 57, 181, 189, 266, 292, 314] for reviews) and has since been described for a number of animal cellular and viral mRNAs [27, 257, 342, 343]; it may also occur in yeast [175]. A number of plant viral mRNAs are not capped [366 for review] and, therefore, must have a cap-independent ribosome entry site. Cap-independent translation initiation might still be dependent on ribosome association with the RNA 5' end and not involve a true IRES. For CPMV, three members of the potyvirus

us group, BYDV and STNV, cap-independent translation was shown to be conferred by viral sequences *in vitro* and partially also *in vivo*. In the potyviruses, the important RNA sequences are located upstream of the AUG codon that initiates translation of the polyprotein (TEV [53], PPV [280], PVY [218], TuMV [16]); in BYDV [353] and STNV [74, 334] sequences at the 5' end and near the 3' end of the RNA are required, and in CPMV, the sequence resides between the two first start codons on the M RNA (positions 161 and 512 [333, 350]). Competition experiments suggest that the function of the TEV and TuMV sequences depends on interaction with a cellular factor also involved in cap-dependent translation [16, 53]. The TuMV sequence is active independent of its orientation and, like the CPMV sequence, also downstream of a scanning-inhibiting secondary structure element [16, 333]; however, this structural element reduced the translation by a factor of five for the TuMV leader [16] and expression in the CPMV case was quite low and not different from other internal initiation events on the respective reporter RNA [333]. The effect of the PVY sequence was completely abolished by an antisense oligonucleotide for the first 16 nucleotides, suggesting that the important sequence is close to the RNA 5' end [16]. The cap independence conferred by the TEV leader depends on sequences located more than 80 nucleotides downstream of the 5' end [53]. Efficient, cap-independent initiation with BYDV and STNV RNA required sequences near the 5' end and near the 3' end [74, 334, 353].

The PPV, PVY and CPMV sequences were tested as internal entry sites between two ORFs. With the PPV sequence, only low levels of downstream ORF expression were observed in *in vitro* translation systems, which led to the conclusion that the PPV leader does not act as a true IRES [280]. Expression obtained with the PVY sequence was interpreted as proof for internal ribosome entry [218]; however, the data are difficult to evaluate because no quantitative comparison of downstream ORF translation with translation from a monocistronic RNA was made. The CPMV sequence allowed low levels of downstream ORF translation in the reticulocyte lysate [350], but was tested without success in an animal cell system [23]. In general, caution is required in extrapolating *in vitro* data to the *in vivo* situation. A sequence upstream of the TNV coat protein ORF was found to allow internal ribosome entry in wheat germ extract but not in tobacco protoplasts [240]. *In vitro* translation systems are known to accept also uncapped RNA, depending on the exact

translation conditions like ionic strength and translation factor concentration. The relatively long AUG-less regions that are tested for their IRES potential would direct all initiation capacity by spurious ribosome binding to the next downstream AUG codon and may, therefore, appear more efficient than other RNA sequences. The reported cases of internal ribosome entry on plant viral RNAs seem to need more substantiation.

The leader sequences of the como- and potyviruses are not as long or as complex as those of the distantly related picornaviruses that support internal initiation of translation. There is also no evidence so far that any of the plant viruses inhibits plant translation to create an advantage for translation of its own mRNAs. The picornaviruses inhibit cap-dependent translation by various mechanisms and in the absence of this inhibition viral mRNAs often compete only poorly with cellular RNAs [258]. Plant virus RNAs seem to compete with cellular mRNAs by reducing the requirements for or by increasing the affinity to initiation factors. The leader sequences of many plant viruses (but not all) have been found to increase translation in *in vitro* systems, in transfected plant protoplasts, and in transgenic plants by unknown mechanisms [53, 77, 91, 118, 119, 183, 184, 234, 255, 270, 308, 309, 311, 335, 367] (reviewed by Gallie, this issue). Translation of the uncapped STNV RNA requires eIF 4F at a lower concentration than other RNAs and is not affected by the presence of cap analogues [44], whereas translation of the capped, efficient AMV RNA 4 leader requires less eIF-4F and -4B than, for example, the β -globin RNA, but is clearly cap-dependent [102, 153]. The enhancing effect of the TMV Ω sequence in a yeast extract is independent of the cap-binding factor eIF-4E but still requires eIF-4A (the supposed RNA-unwinding factor) [7], although TMV RNA is naturally capped and Ω lacks strong secondary structure. The TMV Ω sequence may act differently to other translation enhancing plant viral RNA leaders since it is active in *E. coli* and almost all eukaryotic translation systems [119] (but not or only poorly in monocot cells [120]). The activity of Ω is end-dependent in eukaryotic translation systems [197], and two sequence motifs are crucial in plant protoplasts [126]; for the activity in *E. coli* the 5' end with homology to a recently defined sequence element that can functionally replace a normal Shine-Dalgarno sequence may be responsible [121, 177]. No particular sequence motifs with enhancing effect have been found in other plant viral RNA leaders. For a more detailed review of these features see the contributions of D. Gallie (this volume).

It is also likely that some cellular mRNAs have leader sequences which modulate translation due to their particular translation factor requirements. For example, the untranslated regions of the barley α -amylase specifically enhanced translation in aleurone cells [129]. Other candidates for such leader sequences are the mRNAs translated under stress or developmental conditions that are detrimental to the translation of most other cellular mRNAs, and where discrimination occurs at the level of translation initiation ([127] for review).

In other eukaryotes, leader sequences and also 3'-untranslated sequences can modulate translation and RNA stability in concert with mRNA-specific proteins ([235, 316] for reviews); no example of such a mechanism has been described so far for plants.

Effect of the 3'-untranslated region

All the examples described above demonstrate flexibility in the efficiency and location of translation initiation. Somewhat surprisingly, the 3'-untranslated region (including the poly(A) tail) alone or in conjunction with the 5' leader can have a positive influence on translation initiation (reviewed by Gallie, this issue). These effects of the RNA 3' ends are probably commonplace but as they have been studied in only a few cases are treated here as exceptions. The mechanism for the enhancement is not known, but it is probably similar for the poly(A) tail and for the non-polyadenylated 3' ends of some RNA viruses [122, 125, 288]. In yeast, effects of the poly(A) tail are effective on the formation of the 80S ribosome [251, 290] and are mediated by the poly(A)-binding protein (PABP) [180, 250 for reviews]. The importance of the PABP was also observed in a pea *in vitro* translation system [304]. In plants, sequences far away from the RNA 5' end in conjunction with 5' leaders can cause cap-independence or at least reduce cap-dependence, as shown for STNV [334], TMV [370], BMV [191] and BYDV [353]; this suggests involvement in ribosome binding to the RNA. The stimulatory effects of 3'-UTRs seem to be quite variable in different assay systems and apparently are not always reproducible (e.g. the effects of the 3'-UTR of BMV described by Gallie and coworkers [128, 217] could not be reproduced by Lahser *et al.* [216]). The effects of the poly(A) tail in transfected plant protoplasts were much stronger in the presence of a 5' cap than in its absence. Exogenously added poly(A) stimulated translation of capped, non adenylated mRNAs in yeast [251] and inhibited that

of uncapped RNA in yeast [251] and in plant extracts [130], suggesting that poly(A) interacts with factors required for translation and that it may form a complex with the capped 5' end of the RNA, which is particularly efficient in ribosome binding (reviewed in detail by Gallie, this issue). In the presence of PABP, an oligo(A) tract in the mRNA leader is inhibitory for translation [81]. In *Xenopus* oocytes the poly(A) tail was found to enhance translation reinitiation [117], which means that in this case it acts after the first round of translation of an ORF on ribosomes that have translated the ORF already once. It is unclear how such a recycling of ribosomes could occur, but polysomal RNAs with a circular appearance have been observed in a variety of cases [64, 92, 171, 215], and sequence interactions between the ends of plant viral RNAs have been observed or suggested [74, 75, 103, 319, 334]. This interpretation has similarities with the ribosome shunt. It has been noted that the ribosome shunt *in vivo* might also serve for such a recycling since in the pararetroviral RNAs the shunt donor site is also present in the terminal redundant part at the 3' end of the RNA [114]. A ribosome recycling might help to increase the density of ribosomes on RNAs that can be successfully translated. In most present day translation studies, kinetics of polysome loading are not considered and only the amount of synthesized protein after the incubation period is measured. Also, in most of the studies of the effect of poly(A) tails or 3'-untranslated regions in yeast or plant systems, no discrimination between first initiation and re-initiation was made, and the kinetics of protein production with the different leader and 3'-untranslated regions still has to be investigated. Loading of mRNAs into large polysomes can be considerably slower than the transit time of a translating ribosome on such an mRNA [254], which suggests that it is not determined by primary binding of ribosomes to an RNA, but by rebinding of ribosomes that have translated already once. Reinitiation is also differently affected by initiation inhibitors and, therefore, may be functionally different from first initiation events [254]. While the effect of a cap structure on translation efficiency in transfected plant protoplasts was evident at the earliest measured time point, the full effect of the poly(A) tail was apparent only later [120]. Reinitiation may also be less or not cap-dependent, which may be one explanation why in some cases the 3'-UTR together with some 5' sequences conferred cap independence (see above). If reinitiation is partially involved in the mechanisms of translation enhancement by 3' regions, it is to be expected that these effects will differ with

different reporter genes and possibly also with the precise linkage of the reporter ORF to the 3'-untranslated region, since in this case the mode of translation termination and subsequent ribosome migration should be important.

In other eukaryotes, 3'-UTRs of specific mRNAs have been found also to contain a number of other signals involved in RNA transport and localization, specific translation repression, and translation-dependent or independent RNA degradation ([79] for review). Strong differences in gene expression levels in plants have been described for reporter constructs with different 3'-untranslated regions [176], but the cause of the effect (translation, 3'-end processing, RNA stability, transport or localization) has not been elucidated.

Cotranslational effects

The translation process is in general precise and competing events such as misreading by 'wrong' tRNAs or shifts in the reading phase occur with a significant frequency only if decoding of the codon at the A site by a cognate tRNA or a termination factor is slow or disturbed [11]. In bacteria, translating ribosomes have been found to even skip longer stretches of the RNA completely [25, 172, 357]. This extreme is so far unknown in eukaryotes but smaller 'programmed errors' occur during translation elongation. The paradigm for such processes in eukaryotes is frameshifting and stop codon suppression in retroviruses and retrotransposons, which use both mechanisms to express their *pol* gene as a fusion protein with the upstream capsid proteins in a stoichiometrically controlled ratio ([156, 286] for reviews). A similar mechanism is also used by other viruses of mammals [40, 182] and yeast [341], but not by the plant pararetroviruses, which are relatives of the animal pararetroviruses [167, 286]. In these viruses, the *pol* ORF is either translated separately from the upstream, overlapping ORF corresponding to the retroviral *gag*-ORF [297] or is part of a long precursor ORF that also contains the *gag* functions [286]. Instead, frameshift events have been detected or proposed for *pol* gene expression of luteo- [85], enamo- [82], diantho- [195, 287], sobemo- [230] and carlaviruses [141]. Frameshift may also occur in the BWYV ST9-associated RNA [61] and in closteroviruses [4, 190]. In all cases except the last, frameshift occurs leftwards to the -1 reading phase (Table 3).

The frameshift sites of BYDV-PAV [39, 85, 132], two strains of PLRV [214, 274], RCNMV [195] and CfMV [230] have been studied in greater detail. Like

the frameshift signal of retroviruses and many retrotransposons they consist of a 'shifty' heptanucleotide sequence of the type X.XXY.YYZ (arranged in codons of the 0 frame) and a close downstream secondary structure element (Table 3). The sequences of a number of retroviral transframe-proteins have been determined and on this basis it has been suggested that the frameshift occurs when the peptidyl tRNA at the XXY codon at the P site and the aminoacyl RNA at the YYZ codon at the A site simultaneously slip backwards one nucleotide to the XXX and YYY codons, respectively [178]. Frameshifting does not necessarily lead to a unique transframe protein but rather to a small number of variants with slightly different amino acid sequences around the frameshift site [179, 356]. Frameshifting requires that the two tRNAs at the A and the P sites can stably interact with the new codons with mismatches only at the wobble position. It is caused or enhanced by ribosome pausing due to the downstream structural element [60, 313, 329, 330, 336] and can be influenced by the translation frequency [169]. The downstream element can be a simple hairpin structure or an elaborate pseudoknot [56, 301, 329, 330]. The nature of the codons (or their cognate tRNAs) is also important: of all possible YYZ codons, so far only AAC, UUU, UUA and recently AAU have been found [156, 243, 329].

That most of the studied frameshift events in plants follow the same mechanism was elucidated by determination of the sequence of the BYDV transframe protein [85] and by mutation analyses of the shifty heptanucleotides and the supposed downstream pause elements [132, 195, 214, 274, 363]. Frameshift sites were analyzed either in their natural contexts or by inserting the sites into a reporter ORF. Depending on the frameshift signal and the assay system, frameshift efficiencies of 1–30% were observed. High efficiencies were obtained with the Polish strain of PLRV and with CfMV in *in vitro* translation systems [214, 230], while the other signals direct frameshifting at only 1–4% efficiency in different *in vitro* systems or in plant protoplasts. Signals consisting of A and U nucleotides tend to produce higher efficiencies than those containing G or C [141, 274]. The BWYV ST9-associated RNA may contain a particularly efficient frameshift site, which has, however, not yet been identified [61].

A stop codon found immediately downstream of many of the shifty sites has a positive effect in some assay systems, probably because it contributes to ribosome pausing [39, 85]. In wheat germ extract, the BYDV frameshift event is greatly stimulated by a

Table 3. Frameshift. Sequences at proven or suggested sites in plant viral mRNAs. Virus acronyms and the family are given. The frameshift site is arranged in the 0 reading frame. For the -1 frameshift sites, the given sequence starts with the first nucleotide of the shifty site (X.XXY.YYN) and ends at the beginning of the downstream pause signal. Data are compiled from the indicated references; if no reference is given, data are from the compilation by Miller *et al.* [243].

	Frameshift site	Pause signal	Reference
-1 shifts			
<i>Luteoviruses subgroup 1</i>			
BYDV-PAV	G.GGU.UUU.UAG.AGG	hairpin or pseudoknot	243
SDV	G.GUU.UUU.UAG.AGGG	hairpin	
<i>Luteoviruses subgroup 2</i>			
PLRV-G	U.UUA.AAU.GGG.ACA	hairpin	274
PLRV	U.UUA.AAU.GGG.CAA	pseudoknot	132, 214
BWYV	G.GGA.AAC.GGG.AAG	pseudoknot	132, 349
BYDV-RPV	G.GGA.AAC.GGG.AAG	pseudoknot	
<i>Enamovirus</i>			
PEMV RNA2	U.UUU.UGG.UAG	hairpin	
PEMV RNA1	G.GGA.AAC.GGA.UUA.U	pseudoknot	
<i>Dianthovirus</i>			
RCNMV	G.GAU.UUU.UAG.GCG	hairpin	195
CRSV	G.GAU.UUU.UAA.GU	hairpin	287
<i>Sobemovirus</i>			
CFMV	U.UUA.AAC.UGC.CAG.CG	hairpin	230
SBMV	U.UUA.AAC.UGC.UUG.CG	hairpin	230
RYMV	U.UUA.AAC.UGC.CAG.GG	hairpin	230
<i>Carlavirus</i>			
PVM	U.AGA.AAA.UGA	none?	141
$+1$ shifts (proposed)			
<i>Closterovirus</i>			
BYV	CGG.GUU.UAG.CUC	pseudoknot	4
CTV	CGC.GUU.CGC	none?	190

sequence located more than 3 kb downstream of the site [353], and sequences 40–100 nucleotides upstream of the site may also be required [246].

The frameshift efficiency of a given signal is not only determined by the *cis*-acting sequences but also by the available tRNAs. In infected *Nicotiana benthamiana* plants, shift-promoting tRNA (classes) exist that decode UUU, UUA, UUC and the asparagine codon AAU but none that decode the asparagine codon AAC [195].

The supposed frameshift signal of PVM differs from those described above. The shifty sequence U.AGA.AAA.UGA allows only one basepair to be formed after the shift between RNA and tRNA at the P site, which is hardly sufficient to drive the shift. It was, therefore, suggested that in this case the shift

occurs after peptidyl transfer and translocation when the last codon of the ORF (AAA) together with the peptidyl tRNA occupies the P site and the ribosome awaits recognition of the stop codon at the A site by the release factor [141]. This model was supported by mutagenesis analyses of the role of the stop codon and of the preceding A stretch. The frameshift signal was less effective than those described above (about 0.3% in the reticulocyte lysate) and the postulated frameshift product was not detectable in infected plants. It is noteworthy that the downstream ORF, which should be linked by the frameshift event to the upstream coat protein ORF, has its own translation start codon and is also translated separately (at least *in vitro*) by an unknown mechanism [141].

A number of yeast retrotransposons use a +1 frameshift to produce the correspondent of a *gag-pol* fusion protein [22]. In the case of Ty3, the sequence GCG.AGU.U is decoded as Ala-Val by reading the underlined codons. No slippage of the tRNA^{Ala} is possible but the frameshift efficiency depends on the presence of the 'rare' AGU codon and certain features of the tRNA that decodes the GCG codon or (more general) other special codons located upstream of a rare codon. Artificial frameshift sites can be constructed by combining codons that are decoded by such 'shifty' tRNAs with rare codons [261, 351]. A similar codon configuration may be present in a suggested +1 frameshift site between closterovirus ORFs 1a and 1b, which contains a rare CGG codon (CTV [190]) or a stop codon (BYV [4]) and, at least for CTV, lacks obvious secondary structure [190]. Frameshifting has still to be proven for these viruses.

The mammalian type C retroviruses use stop codon suppression instead of frameshifting to produce the *gag-pol* fusion protein [97, 156, 365]. Suppression occurs with an efficiency of about 5% *in vitro* and *in vivo* and is accomplished by misreading of an UAG termination codon by a glutaminyl tRNA [97]. A UGA stop codon at the same position is decoded as arginine, cysteine or tryptophan [97]. Since the stop codons of normal cellular genes are not suppressed detectably under the same conditions, it was concluded that a specific context is responsible for the effect in retroviral RNAs. The presence of a pseudoknot structure 8 nucleotides downstream was required for efficient readthrough *in vitro* [98, 360, 361], while *in vivo* only evidence for the requirement of a stem structure was obtained [96].

Stop codon suppression has been suggested as a mechanism for the expression of some seed storage proteins whose ORFs are interrupted by stop codons [93, 223, 352]. It is, however, not always clear whether these ORFs are on functional mRNAs or whether they represent silent pseudogenes. It has been pointed out that storage protein genes contain a high number of CAA and CAG codons (coding for glutamine) which could mutate into a stop codon by a simple C-to-T transition [93]. A stop codon in the hordein gene λ -hor1-14 was suppressed in bombarded barley endosperm with an efficiency of only 0.6% [93].

A large number of plant RNA viruses use stop codon suppression to produce components of RNA dependent RNA-polymerases or elongated coat proteins that are probably required for transmissibility by their respective vectors [243, 326, 366]. Unlike

for retroviruses, no structure requirements for stop-codon suppression have been observed in plant systems. Rather readthrough is dependent on the immediate sequence context of the stop codon and on the presence of certain tRNA species that can decode such stop codons as sense codons (Table 4). Suppressible stop codons have been inserted into GUS ORFs and readthrough has been quantified in transfected plant protoplasts. The context of the stop codon of the tobacco mosaic virus 126 kDa ORF (CAA.UAG.CAA.UUA) allowed particularly efficient (around 5%) readthrough for all three possible stop codons [306, 307, 346]. The leakiness of this stop codon context was used to express angiotensin-converting enzyme inhibitor peptide as the C-terminal extension of a subfraction of TMV coat proteins from an accordingly engineered infectious TMV clone [148]. Mutagenesis analysis of the readthrough sequence defined (C/A)(A/C)A.UAG.CAR.YYA (with R=purine, Y=pyrimidine) as optimal consensus. This consensus sequence, like the UGA readthrough signals of TRV [150], PEBV [229], PCV and SBWMV [163] and of sindbis virus RNA [219], fits to the statistical analysis of eukaryotic stop codon contexts: efficiently recognized stop codons normally avoid a C directly downstream but rather have a purine in this position [9, 42, 43, 236, 344]. In the light of this finding, the readthrough of the UAG.G signals of the carmo- and luteoviruses, and of the tombusviruses [159, 296] (Table 4) has to be functionally different and may require additional *cis*-acting sequences or specific tRNAs. The presence of a conserved CCCCCA motif or repeated CCXXXX motifs downstream of the luteo- and carmovirus readthrough sites has been noted but the involvement in readthrough has not been experimentally tested so far [243]. The signals of TRV, CarMV, MCDV, BYDV and BWYV were active in *in vitro* systems or in infected plants [12, 52, 100, 265] but were rather ineffective in transfected tobacco protoplasts [306].

The sequences of plant viral readthrough proteins produced *in vivo* have not been determined, but it is likely that the stop codons are decoded by particular tRNAs, as has been found in *in vitro* systems and *Xenopus* oocytes, where addition of tRNA^{Tyr} with a G Ψ A anticodon but not with a GUA anticodon greatly stimulated readthrough of the TMV signal [15, 20, 21, 368]. Interestingly, this tRNA is rare in young wheat leaves but is abundant in older tissue, suggesting that processes like readthrough (and possibly also frameshifting) are developmentally regulated. The

Table 4. Leaky termination codons of plant virus ORFs. The immediate sequence context of partially suppressed termination codons (underlined) and the protein resulting from translational suppression are listed. References to those viral sequences not further mentioned in this review can be found in the compilation by Miller *et al.* [243].

	Read-through stop codon	Read-through protein	References
<i>Tobamovirus</i>			
TMV	CAA. <u>UAG</u> .CAA.UUA	replicase	264, 306, 346
<i>Tymovirus</i>			
TYMV	CAA. <u>UAG</u> .CAA.UCA	polyprotein extension	247, 38
<i>Furovirus</i>			
BNYVV	CAA. <u>UAG</u> .CAA.UUA	coat protein extension	35, 373
<i>Luteovirus</i>			
BYDV	SCC.AAA. <u>UAG</u> .GUA.GAC	coat protein extension	243
PLRV	CCC.AAA. <u>UAG</u> .GUA.GAC	coat protein extension	
BWYV	CCC.AAA. <u>UAG</u> .GUA.GAC	coat protein extension	
SDV	GCU.AAA. <u>UAG</u> .GUA.GAC	coat protein extension	
BWYV-ST9	AAA. <u>UAG</u> .GGC	replicase	
<i>Enamovirus</i>			
PEMV	UCC.CUC. <u>UGA</u> .GGG.AC	coat protein extension	
<i>Carmovirus</i>			
CarMV	AAA. <u>UAG</u> .GGG	replicase (1. stop)	
	CAG. <u>UAG</u> .UUG	replicase (2. stop)	
MCMV	AAA. <u>UAG</u> .GGG	replicase	
CCFV	CGC. <u>UAG</u> .GGG	replicase	
MNSV	AAC. <u>UAG</u> .GGG	replicase	
TCV	CGC. <u>UAG</u> .GGG	replicase	
<i>Necrovirus</i>			
TNV	AAA. <u>UAG</u> .GGG	replicase	68
<i>Tobravirus</i>			
TRV	UUA. <u>UGA</u> .CGG.UUU	replicase	150
<i>Tombusvirus</i>			
TBSV	AAA. <u>UAG</u> .GGG	replicase	159, 296
<i>Sobemovirus</i>			
PCV RNA1	AAA. <u>UGA</u> .CGG	replicase	163
SBWMV RNA1	AAA. <u>UGA</u> .CGG	replicase	303
SBWMV RNA2	AGU. <u>UGA</u> .CGG	coat protein extension	

TMV readthrough site was also efficiently suppressed (20%) in transfected tobacco protoplasts by a bean tRNA^{leu} with an artificially introduced CUA anticodon [51] and in protoplasts and transgenic plants (up to 10%) by modified trp tRNAs [104]. The fact that such suppressor tRNAs can be overexpressed without strong phenotypic changes suggests that normal stop codons are not significantly suppressed by these tRNAs. It has, however, been noted that suppression is more efficient in protoplasts than in plants, suggesting that only

transgenic plants are regenerated which have a lower expression level of the suppressor tRNA [51].

The leaky UGA stop codon of TRV is suppressed by a tRNA^{Trp} with a CmCA anticodon [369]. One such tRNA originated from the chloroplast and was more efficient than that from the cytoplasm. Since the respective chloroplast and mitochondrial tRNAs are almost identical and TRV appears to be associated with mitochondria in infected cells it is possible that the virus uses the mitochondrial tRNA as suppressor

in vivo [369]. At present it is unknown which tRNA suppresses the UAG.G stop codon of the luteoviruses.

Different suppressing tRNAs could be the reason that readthrough efficiencies vary with the assay system, but possible involvement of *cis*-active sequences distal to the readthrough sites may also account for the variation. For example, in *in vitro* translation experiments with the full-length TMV RNA, fourfold higher readthrough efficiencies have been obtained [264] than with the artificial constructs of Skuzeski *et al.* [306, 307], and it has to be determined whether this is an effect of more distal *cis*-acting sequences or of the different assay systems.

In other organisms, reading of UGA stop codons as selenocysteinyl codons is a special type of stop codon suppression ([95, 157] for reviews). Selenocysteine has been found in plant proteins [41] and a selenocysteine-tRNA recognizing UGA has been identified in *Beta vulgaris* [157]. However, it is unknown whether UGA misreading is the only way of selenocysteine incorporation into plant proteins and whether this process requires additional *cis*-active sequences on the mRNA, as was found in animal cells [30, 302] and *E. coli* [160].

For retroviruses, it is still a matter of discussion whether virus infection alters the frameshift or stop codon suppression capacity of the cell. Controversial results on induction of synthesis of shifty tRNAs or on differences in the levels of tRNA modifications with consequences for the frameshift efficiency have been presented ([156] for review). For plants, however, nothing comparable is known for tRNAs involved in stop codon suppression, although concentration differences in different tissues have been reported [21, 285], and the findings of Kim and Lommel [195] may indicate that the frameshift-prone tRNAs may also be represented differently in different plants.

Translation under special conditions

Many responses of plants to developmental and environmental signals or to stresses like wounding, heat shock, lack of water or oxygen have post-transcriptional components (for reviews see Gallie, this issue; [127, 143, 320]). Generally the translation machinery concentrates on a number of specific mRNAs while others are not translated anymore and are either stored as inactive RNAs or degraded. The pattern of polysome-associated mRNAs and overall translational activity can vary widely, but little is known about the fate of specific mRNAs and about the controlling *cis*- and *trans*-acting features. Translational

control is exerted mainly at the levels of initiation [29, 277] and elongation [10, 28, 99, 248, 305, 310, 348, 354]. Heat-shock mRNAs are more competitive under heat stress and this feature has been attributed to their leader sequences [131, 269, 293]. These sequences are, however, very heterogeneous and no common motifs have been discerned so far [187].

The association of mRNAs with architectural components of the cell or the precise location of an mRNA in the cytoplasm can influence the translational activity on that RNA [67, 149]. An alteration of such association or sequestration seems to be one level of translational control under conditions of stress or changes in developmental programs in plant cells [10, 13, 24, 70, 256, 277], but the molecular basis of this phenomenon is unclear.

Conclusions

A number of features that modulate translation have been detected in mRNAs translated in animal and yeast cells. Some of these are also found in RNAs translated in the cytoplasm of plant cells, predominantly on viral mRNAs. Quantitative control of the synthesis of two (or more) proteins from one mRNA is achieved by leaky scanning, frameshifting or stop codon suppression, or transactivated reinitiation on plant viral mRNAs. With the possible exception of stop codon suppression, it is not known whether any of these mechanisms is active also in the control of nuclear gene expression or is so plant, tissue, or development specific that it could be part of the host-range control of plant viruses. The 5' leader sequences and the 3'-untranslated region separately or in conjunction increase the cap-dependent or -independent affinity for ribosomes and probably can serve as a paradigm for cellular RNAs that are translated under special conditions. Short ORFs are found in the leaders of a number of nuclear and viral genes, but it is unknown whether these sORFs simply serve to reduce downstream translation or whether they regulate translation in response to cellular conditions. Further work is required to link these features to the translation control events observed in plant cells in response to a variety of stresses and developmental or environmental signals.

In particular, it is to be expected that further study of the influence of 5' leaders and 3'-UTRs on (cap-independent) translation, of the translation factors involved in this process in plants, and further analysis of the unusual translation mechanisms of the plant

pararetroviruses will contribute essential knowledge to our understanding of eukaryotic translation processes.

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