TWO LEAKY TERMINATION CODONS IN AMV RNA 1

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1. Introduction

Alfalfa mosaic virus (AMV) is a plant virus with a tripartite genome [1,2]. Each of the genomic RNAs (RNA 1, 2 and 3; M_r 1.04, 0.73 and 0.62 × 10^6, respectively, [3] contains mainly unique information [4] and has the plus polarity. Besides the genomic RNAs virus preparations contain a subgenomic RNA (RNA 4; M_r 0.25 × 10^6 [3], coding for the viral coat protein [5–8].

The products directed by AMV-RNAs 1, 2, 3 and 4 in several cell-free systems have been studied [7–14]. Under all conditions the main product directed by AMV-RNA 2, 3 or 4 is the same. However, depending on its concentration AMV-RNA 1 directs either a 115 000 M_r protein (at low mRNA concentration) or a mixture of 58 000 M_r and 62 000 M_r proteins (at high mRNA concentration) in the rabbit reticulocyte cell free system. At mRNA concentration around the optimum of incorporation all 3 proteins are formed [14]. These 3 proteins represent overlapping peptide chains with identical N-termini. The formation of partial translation products under the direction of AMV-RNA 1 was attributed to the limited availability of the succeeding tRNA(s) (suppressor or isoacceptor), since addition of excess of wheat germ tRNA or amino acids to the cell free system resulted in the suppression of internal termination [14].

For several other plant viruses (tobacco mosaic virus (TMV) [15], tobacco rattle virus (TRV) [16], turnip yellow mosaic virus (TYMV) [17], cowpea mosaic virus (CPMV) [18–20], potato virus X [21] and southern bean mosaic virus [22]) it has been reported that translation in vitro of one purified RNA species yields ⩾ 2 proteins. For TMV [15], TRV [16], TYMV [17] and CPMV [19] it has been shown that the smaller proteins are identical to the N-terminal part of the larger products. It is possible that the formation of these smaller proteins is an artefact of the in vitro system used. However, it is also possible that both the full-length as well as the smaller proteins are functional products and that the relative amount of the different products from the same messenger are regulated by the use of leaky termination codons. Such a situation has been described for TMV, TRV and TYMV RNAs [15, 16,23,29].

Here we show that suppression of the formation of smaller products under the direction of AMV-RNA 1 can be performed by a tRNA, which can be charged with glutamine and which is present in wheat germ. Furthermore evidence is presented that this glutamine tRNA is a suppressor tRNA, probably translating the UGA stop codon.

2. Materials and methods

AMV-RNA was obtained from the purified nucleoprotein components of AMV strain 425 as in [8,12]. Protein synthesis was done in the mRNA-dependent rabbit reticulocyte cell free system. Preparation of the lysate and the mRNA-dependent cell free system as in [14]. A standard 35 μl reaction mixture contained: 68% (v/v) mRNA-dependent rabbit reticulocyte lysate, 17 μM hemin in 90% ethyleneglycol containing 50 mM Tris–HCl adjusted to pH 8.0, 26.5 μg creatine kinase/ml, 10 mM creatine phosphate, 70 mM KCl, 20 μM each of 19 unlabelled amino acids, 0.67 mM $CaCl_2$, 60 units micrococcal nuclease, 1.34 mM EGTA, 25 μg wheat germ tRNA/ml, 1 μCi L-[^{35}S]methionine (spec. act. 1090 Ci/mmol) and various amounts of mRNA. [Mg^{2+}] and [K^+] used were optimal for protein synthesis. Variations are mentioned in the legends. Incubations were performed at 30°C for 60 min. Total incorporation of label was checked by trichloroacetic acid precipitation as in [14]. Analysis of the polypeptides was done on 11% polyacrylamide slab gels containing 0.1% SDS as in [24], followed by

staining and fluorography as in [25].

L-[^{35}S]Methionine was from the Radiochemical Centre Amersham; L-[^{3}H]glutamine from New England Nuclear Corp.; hemin from Sigma; micrococcal nuclease (EC 3.1.4.7.), creatine kinase (rabbit muscle) and creatine phosphate (disodium salt) from Boehringer; X-O-mat Kodak films were used for fluorography; paromomycin was kindly provided by Dr J. Davies, University of Wisconsin-Madison, WI.

3. Results and discussion

3.1. *Suppression of termination by excess of glutamine*

We had shown [14] that under certain conditions only the 5′-half of AMV RNA 1 is translated, resulting in the formation of 2 overlapping proteins (M_r 58 000 and 62 000, respectively). From the fact that there are hardly any products larger than the 62 000 M_r protein it follows that the second stop is less leaky than the first one. The internal stops could be suppressed by the presence of excess wheat germ tRNA or amino acids during protein synthesis. To find out which tRNA species is (are) responsible for the inhibition of elongation beyond the C-terminus of these two proteins, we studied the effect of an excess of each of the 20 amino acids separately (fig. 1 (1–4)). Only in the presence of glutamine is the incorporation of amino acids much higher (191 000 vs 92 000 cpm), and there is an increase in the formation of the 115 000 M_r protein, indicating that the same tRNA is required to pass both stop points. As expected excess of glutamine had no effect on the pattern of products directed by the other AMV RNA (not shown).

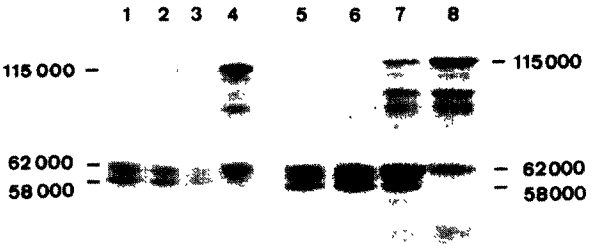

Fig.1. Effect of excess of each of the 20 amino acids on the nature of products directed by 100 μg AMV RNA 1/ml in the presence of optimal (25 μg/ml) (1–4) and suboptimal (5 μg/ml) (5–8) concentration of wheat germ tRNA. (1,5) Controls; (2,6) 0.5 mM of each of the 20 amino acids except tryptophan and glutamine. The effect being identical only one lane (Asn) is shown; (3,7) 0.5 mM tryptophan; (4,8) 0.5 mM glutamine; (1–4) and (5–8) are from different experiments.

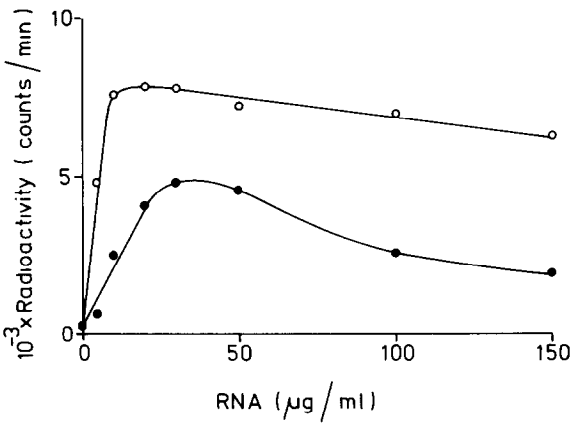

Fig.2. Incorporation of [^{3}H]glutamine into proteins synthesized under the direction of increasing concentrations of AMV RNA 1 (•) and AMV RNA 4 (○).

3.2. *Why is glutamine limiting the full-length translation of AMV RNA 1 ?*

There can be several reasons why the formation of full-length products under the direction of AMV RNA 1 is dependent on a high concentration of glutamine.

(1) Glutamine or glutamine tRNA is limiting

In the latter case the effect of an excess of glutamine is due to the presence of a higher percentage of aminoacylated glutamine tRNA. If this explanation is correct, the amount of glutamine incorporated under the direction of other AMV RNA would always be less or equal to that incorporated under the direction of AMV RNA 1. Fig. 2 shows that on the contrary under the direction of AMV RNA 4 about twice as much glutamine can be incorporated as under the direction of AMV RNA 1. The possibility that only one of the tRNAs for glutamine is present in limiting amounts, is also contradicted by the results obtained with AMV RNA 4. The complete base sequence of this RNA is known [26] and in the coding part of the RNA the code word CAA is present 6 times (corresponding to

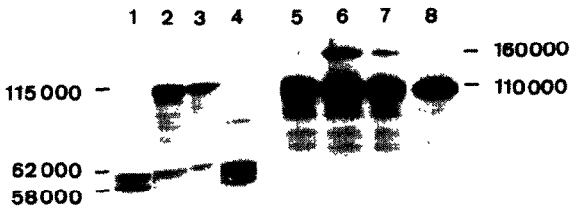

Fig.3. Stop and read through of the translation of AMV RNA 1 and TMV RNA. Products directed by 100 μg/ml AMV RNA 1 (1–4) and by 100 μg/ml TMV RNA (5–8), in the presence of 10 μg/ml paromomycine (2,6), 0.4 mM magnesium (3,7), 0.4 mM spermidine (4) and 0.5 mM glutamine (8). (1,5) controls; (1–4) and (5–8) are from different experiments.

amino acid position 4, 28, 55, 128, 156 and 158 in the viral coat protein [27]) and the code word CAG 3 times (corresponding to 19, 49 and 175 [27]). As mentioned above AMV RNA 4 is always translated into full-length product.

(2) Glutamine is contaminated with Mg^{2+}

Supraoptimal concentrations of Mg^{2+} will also induce read through (TMV [15] and TRV [16] and fig. 3 (3)). This explanation can be ruled out since addition of glutamine had no effect on the products directed by TMV RNA while addition of Mg^{2+} had the expected effect (fig.3 (7,8)).

(3) The incubation mixture contains besides the normal tRNAs for glutamine another $tRNA^{Gln}$ which can translate one of the 3 stop codons and about halfway AMV RNA 1 there are 2 leaky termination codons.

This situation is similar to that described for TMV RNA (leaky amber [15]), TRV RNA (leaky opal [16]) and TYMV RNA (leaky amber [23] and leaky opal [29]).

3.3. *Code word and source of suppressor $tRNA^{Gln}$*

The genomic RNA of TMV can be translated into 2 large proteins (M_r 110 000 and 160 000). The synthesis of the 2 proteins is initiated at the same site, and the larger product is generated by suppression of an amber (UAG) termination codon. In accordance with the wobble hypothesis [28] suppression can also be achieved by ochre (UAA) suppressor tRNA [15]. If the suppressor $tRNA^{Gln}$ recognizes the code words UAG or UAA a high concentration of glutamine will induce more read through on TMV RNA. However, the pattern of products directed by TMV RNA is not changed by the addition of excess glutamine (fig. 3 (8)), suggesting that these codons are not recognized by the suppressor $tRNA^{Gln}$.

Polyamides spermine and spermidine specifically enhanced the efficiency of suppression of termination of the UAG codon by eukaryotic tRNA in [29]. Spermidine enhances the incorporation under the direction of a high concentration of AMV RNA 1 (165 000 vs 92 000 cpm) but has only a small effect on the pattern of products synthesized (fig. 3 (4)).

Taken together these results suggest that the suppressor $tRNA^{Gln}$ will translate the UGA stop codon.

Our standard incubation mixture contains, besides tRNA from rabbit reticulocytes, tRNA from wheat germ. Rabbit reticulocyte lysates contain (besides an UAA suppressor tRNA) an UGA suppressor tRNA, which can be charged with tryptophan [30]. This could suggest that the suppressor $tRNA^{Gln}$ originates from wheat germ.

If our assumption of UGA stop codons in AMV RNA 1 is correct, it is to be expected that the efficiency of suppression will, in the absence of wheat germ tRNAs, be influenced by the concentration of tryptophan. This was not found (not shown). However, this failure could be due to the fact [19] that in the absence of exogenous tRNAs the translation might be hampered by a shortage of several isoacceptor tRNAs. Indeed, in the absence of wheat germ tRNAs more small products were formed, and this will diminish the requirement for suppressor tRNA. To investigate this problem we translated high concentrations of AMV RNA 1 in the presence of varying concentrations of wheat germ tRNA. We found that in the presence of suboptimal concentrations of wheat germ tRNA read through was influenced by the concentration of both glutamine and tryptophan (fig. 1 (5–8)). The fact that at optimal concentration of wheat germ tRNA excess of tryptophan had no effect (fig. 1 (3)) might be due to competition between tRNA species [31].

These results are in accordance with the assumption that AMV RNA 1 contains 2 UGA stop codons and that the UGA suppressor $tRNA^{Gln}$ is present in

wheat germ. Preliminary attempts to purify the suppressor $tRNA^{Gln}$ have revealed that wheat germ contains three $tRNA^{Gln}$ species (Neeleman et al., in preparation). Also in tobacco, which is a host plant for AMV, a suppressor tRNA is present (in preparation).

3.4. *Effect of paromomycin and magnesium on the efficiency of termination*

To see if there is a difference in the termination signals within a cistron and at the end of a cistron we investigated the effect of paromomycin and supraoptimal [Mg^{2+}] on the suppression of termination. The aminoglycoside antibiotic paromomycin causes mistranslation in vitro [32] and phenotypical suppression of all 3 nonsense and several missense mutations in *Escherichia coli* [32]. Supraoptimal [Mg^{2+}] has been shown to increase read through in several cell-free systems [15,16,29,33] probably by diminishing the efficiency of termination. In the presence of paromomycin as well as in the presence of a high [Mg^{2+}] there is a significant increase in the formation of the M_r 160 000 protein under the direction of TMV RNA (fig. 3 (5–7)) and the M_r 115 000 protein under the direction of AMV RNA 1 (fig. 3 (1–3)). However, under these conditions no additional larger proteins are formed. Neither paromomycin nor supraoptimal [Mg^{2+}] had any effect on the size of the products directed by AMV RNA 2, 3 and 4 (not shown).

The results with paromomycin and Mg^{2+} suggest that there is a difference in the stop signals within a cistron and at the proper end. This could be due to the fact that at the end of the cistron several termination codons succeed each other. However, there are also indications that the sequence (and structure) around a termination codon influences the efficiency of termination [15,30,34]. This is also illustrated by the fact that although both termination codons on AMV RNA 1 are suppressed by the same tRNA the efficiency of suppression of the first termination codon is higher than that of the second (fig. 1). The use of leaky termination codons and naturally occurring suppressor tRNAs appear to be a commonly used mechanism in nature, both in prokaryotic and eukaryotic systems [15,23,29,30,34–42].

4. Conclusions

From the results presented above we conclude that:

(i) AMV RNA 1 contains 2 leaky termination codons (probably UGA) which can be suppressed by the suppressor tRNA from wheat germ in the presence of high concentration of glutamine. They can also be suppressed by a suppressor tRNA present in rabbit reticulocytes, in the presence of high concentration of tryptophan.

(ii) The presence of 2 leaky termination codons in AMV RNA 1 makes it possible to generate 3 different proteins starting from a single initiation site. This finding might be connected to the fact that this RNA contains at least 2 complementation groups [42].

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