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TRANSLATION OF ALFALFA MOSAIC VIRUS RNA EFFECT OF POLYAMINES

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1. Introduction

Alfalfa Mosaic Virus (AMV) is a multicomponent virus [1] consisted essentially by four nucleoprotein particles, though other minor components exist of various sizes [2]. Each of the major components contains its own species of RNA [3] with sedimentation coefficients of 24S, 20S, 17S and 12S and corresponding mol. wts. of approx. 1.3×10^6, 1.0×10^6, 0.7×10^6 and 0.33×10^6 [4]. The 12S RNA is not needed for infectivity which can be obtained by inoculation of the mixture of other heavier RNAs and the coat protein [3,5]. Nevertheless, the 12S RNA is found as a structural component of the nucleoprotein particles in the infected cells. These results suggested that the total genetic information is included in the three heavy RNAs in accord with the finding that there is no replicative form for 12S RNA [6] and that there is redundant sequence coding for the coat protein in 17S RNA [7]. Similar results have been obtained with another multicomponent virus, the Brome Mosaic Virus [8,9].

It is obvious that studies on the expression of genes located in such different RNAs by means of in vitro translation should bring some insight into the role of these RNAs and their irregulation.

2. Materials and methods

2.1. *Preparation of wheat germ extracts*

The procedure was derived from that described by Roberts and Paterson [10].

Commerical wheat germ, mechanically extracted and not toasted was supplied by Grands Moulins de Paris (France). Before use, the wheat germ was pre-washed with the grinding buffer: 20 mM Hepes (pH 7.6); 100 mM potassium chloride; 1 mM magnesium acetate; 2 mM calcium chloride; and 6 mM mercaptoethanol. The wheat germ was then ground in the presence of the buffer in a chilled mortar with an equal weight of acid-washed sand of Fontainebleau. The proportion germ/sand/buffer was 1g/1g/5 ml. The grinding was performed gently in a discontinuous fashion for 20 min. The sand and intact cells were eliminated by two centrifugations at 500 *g* for 10 min. The active extract was obtained by centrifugation at 30 000 *g*. The supernatant obtained was made 3.5 mM in Mg^{2+} and the following components were added: 1 mM ATP; 20 μM GTP; 8 mM creatine phosphate; 20 μg/ml creatine phosphokinase; and 2 mM dithiothreitol. This extract was incubated 15 min at 30°C and filtered through a column of Sephadex-25 coarse (50 × 2 cm) equilibrated with the following buffer: 20 mM Hepes (pH 7.6); 100 mM KCl; 5 mM Mg-acetate and 6 mM mercaptoethanol. The excluded proteins were pooled; the usual protein concentration obtained was about 20 mg/ml. These pools, called S_{30} fraction, were stored at −80°C in small separate aliquots.

2.2. *Translational system*

The incubation mixture contained: 20 mM Hepes (pH 7.6); 2 mM dithiothreitol; 1 mM ATP; 25 μM GTP; 7.5 mM creatine phosphate; 20 μg/ml

creatine phosphokinase; 30 μM of each of a mixture of ^{14}C-labelled amino acids with specific activity of 130 μCi/μmole; 3.5 mM magnesium acetate; 80 mM potassium chloride; 4 mg S_{30} extract proteins/ml; polyamines and mRNA as indicated. Incubation was done at 25°C and aliquots were removed at different time intervals for aminoacid incorporation measurements and for product analysis.

2.3. *Plant viral RNA*

The alfalfa mosaic virus, strain S, was multiplied and purified as described previously [4]. Extraction of the four RNAs and their separation on polyacrylamide-agarose gels by electrophoresis have been also described [6]. The individual RNAs obtained were almost homogeneous with maximum contamination of 2–5% from each other.

Tobacco mosaic virus RNA and Brome mosaic virus total RNA were kindly supplied by Dr L. Hirth.

2.4. *Electrophoresis on SDS-polyacrylamide gel*

Polyacrylamide (10%) gel (10 X 13 X 2 cm) was prepared in the presence of 0.4% SDS according to Laemmli [11]. The labelled translational products were detected either by autoradiography on Koderex X-ray films or by fluorography [12] on R. P. Royal X-Omat film.

3. Results

3.1. *Ionic requirements*

In contrast to prokaryotic systems, many eukaryotic cell-free systems for protein synthesis require higher KCl concentration but lower Mg^{2+} concentration. Thus, with the wheat germ system, the optimal concentration for Mg^{2+} was found around 3 mM and that for KCl about 100 mM for various messengers for KCl. In the case of AMV

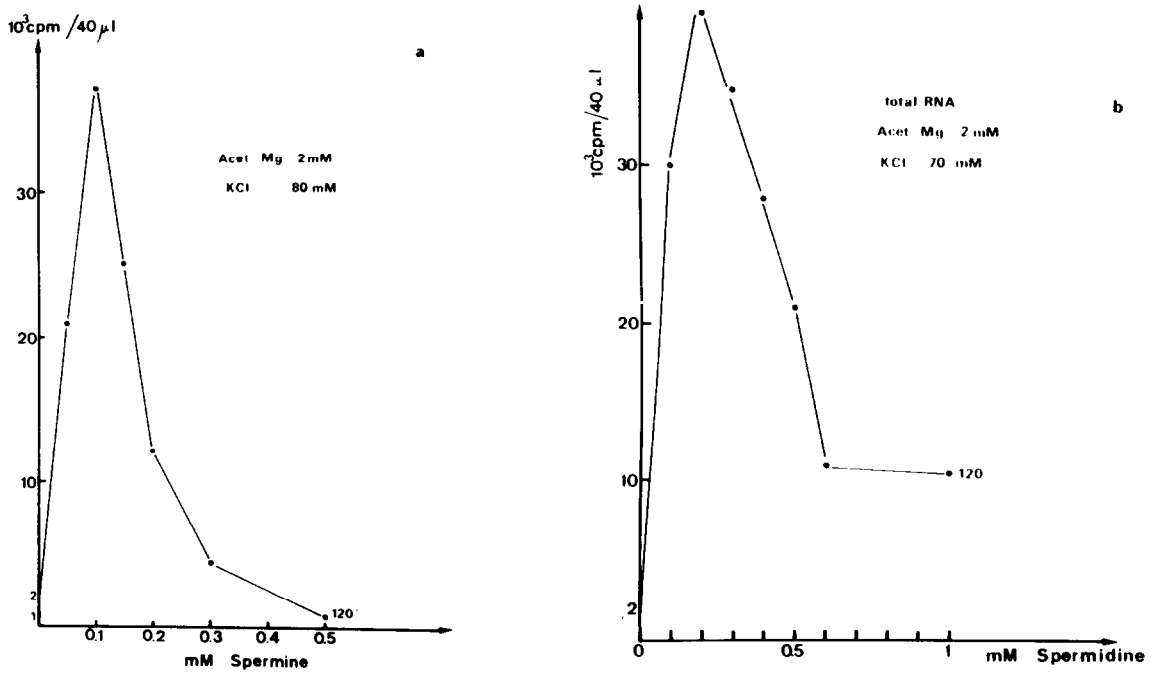

Fig.1. Optimum concentration of spermine and spermidine for translation of AMV RNA. Incubation mixture (100 μl) is the same as described in Materials and methods except that Mg-acetate was at 2 mM; AMV total RNA at 40 μg/ml and polyamines as indicated. Incubation at 25°C for 120 min. Aliquots of 40 μl were spotted onto Whatman 3 MM paper discs which were immerged successively in TCA 10% containing 1% casaminoacids at 25°C for 15 min then in TCA 5% plus casamino acids 0.5% at 90°C for 15 minutes, then three times in TCA 5% and dried in ethanol–ether. Radioactivity was measured by liquid scintillation continuing in PPO - PoPoP toluene system. (a) Spermine. (b) Spermidine.

RNA, we found that in the presence of 3.5 mM Mg^{2+} and 80 mM KCl, all four RNAs were translated at the maximum rate and efficiency though both these values are quite different from one RNA to another [13]. In addition, the initial rate as well as the efficiency can be enhanced by the presence of spermine or spermidine. These polyamines shift the optimum concentration of Mg^{2+} from 3.5 mM to 2 mM without changing the parameter for KCl. Their optimal concentrations are found to lie within very sharp limits (fig.1a and b).

3.2. *Enhancement by polyamines*

Under optimal concentrations of polyamines, the extent of incorporation of amino acids directed by the AMV total RNA is stimulated by 1.5- to 2-fold (fig.2a) as compared with the standard salt conditions. The stimulation of the initial rate of synthesis is even higher because the lag phage observed with the wheat germ cell-free for the translation of many plant viral RNA is almost suppressed by the presence of polyamines. The degree of stimulation of the initial rate depends on the extracts used and the times of storage. For instance, an extract stored at −80°C for 8 months develops a more pronounced lag phase which cannot be suppressed completely by the addition of polyamines (fig.2b). However, the lag phase can also be overcome by a preincubation of the S_{30} extract at 25°C for 15 min just before starting the translation.

The preincubation effect was thus further analyzed with the 105 000 *g* supernatant fraction and ribosomes separately. The results obtained indicate that the stimulation induced by preincubation does not seem to affect directly the ribosomes but rather has its effect on the soluble fraction, as shown in fig.3 by a preincubation of the supernatant fraction in the presence of ^{14}C-labelled amino acids and the mRNA. The lag phase can also be overcome, in the absence of exogenous polyamines, partly with AMV or BMV RNA and entirely with TMV RNA. Addition of spermidine has little effect on a translation mixture make up with a S_{105} supernatant fraction previously preincubated. It is worth noting that remixing the supernatant with ribosomes give a translation

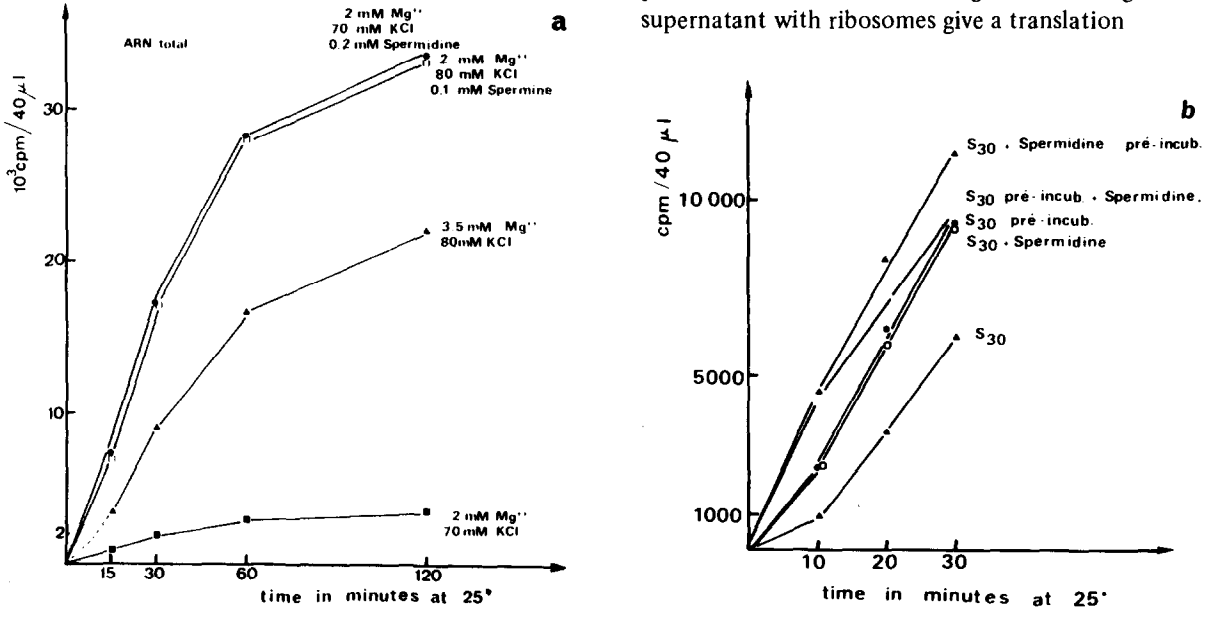

Fig.2. Stimulation of incorporation of amino acids directed by AMV total RNA and suppression of the lag phase by spermine and spermidine. Incubation was performed as described under fig.1. Concentration of Mg^{2+}, KCl and polyamines are indicated. Aliquots of 40 µl of incubation mixture were removed at indicated intervals and treated as described in fig.1. Preincubation of the extract was performed in the complete translation mixture except the viral RNA at 25°C for 15 min and the reaction was started by addition of the messenger. (a) Fresh extract. (b) Extract stored at −80°C for 8 months.

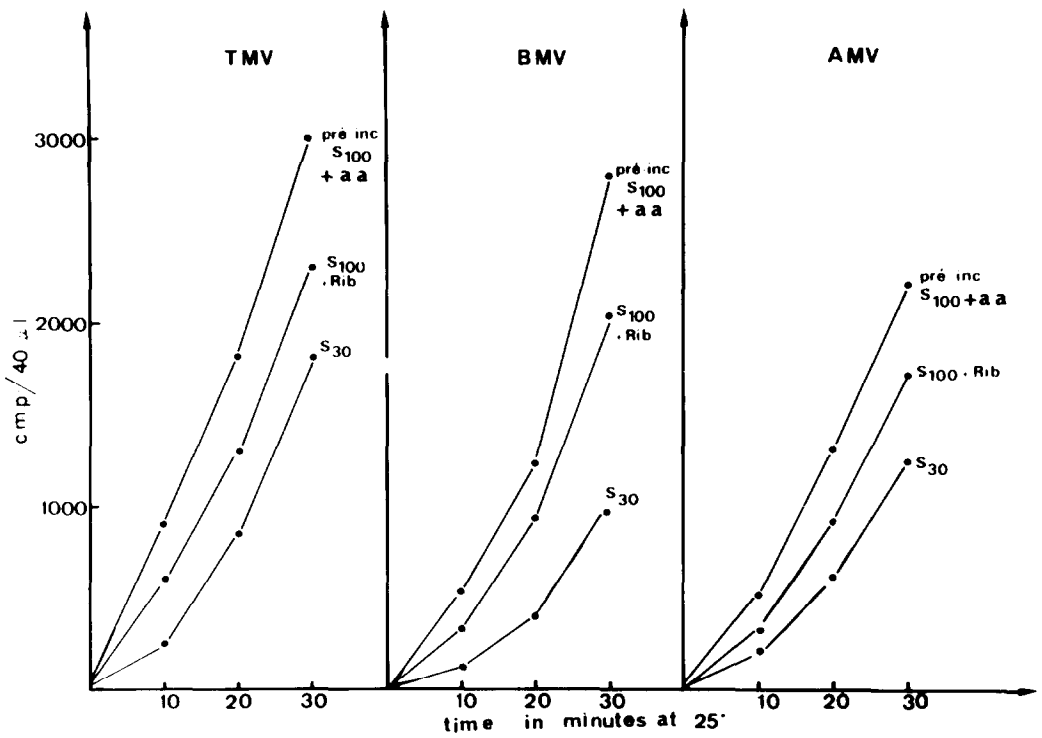

Fig.3. Effect of preincubation of supernatant fraction in the presence of ^{14}C-labelled amino acids on the lag phase. S_{30} extract was centrifuged at 150 000 *g* for 60 min to separate the ribosomes from the supernatant fraction. Two-thirds of the supernatant corresponding to the middle part was withdrawn and kept in ice. The remaining was discarded. The pellet of ribosomes was rinsed once then resuspended in the translation buffer (Hepes-Mg-acetate, KCl-DTT) to make up a volume corresponding to one tenth of the initial S_{30} volume. Preincubation of the supernatant fraction was performed in the complete translation mixture except for the ribosomes at 25°C for 15 min, then cooled in ice. Ribosomes were then added in such a proportion as to restore the initial ratio supernant-ribosomes in the S_{30} extract. The incorporation of ^{14}C-labelled amino acids were started by incubation at 25°C and aliquots were spotted onto paper disks at the time intervals indicated and treated as described in fig.1. Control experiments were carried out with S_{30} extract and the reconstitued extract with S_{150} and ribosomes without preincubation. The concentration of RNA was 50 µg/ml.

mixture more active than the initial S_{30} extract. It appears that the effect on the suppression of the lag phase induced by polyamines may be substituted partly if more entirely by a preincubation of the supernatant or the S_{30} extract. The same suppression has been already observed by Marcus [14] by addition of acylated tRNA and ATP.

With individual purified RNAs spermine and spermidine stimulated to the same extent (about 50--100%) incorporation of amino acids directed by AMV 12S, 20S and 24S RNA. However, for the 17S RNA, the extent is much higher (3- to 5-fold) as shown in fig.4. This case is particularly interesting due to the fact that the translation efficiency under direction of AMV 17S RNA in the wheat germ extract is very low [13]. This relatively low efficiency could not be modified by addition of a great excess of this RNA or of the S_{30} extract. Since 17S RNA is supposed to contain the coat protein cistron [5], the question could be posed of the nature of the polypeptides synthesized in the presence or in the absence of polyamines.

3.3. *Analysis of the translation products*

It is known that the AMV 12S RNA directs the synthesis of the coatprotein in a cell-free system isolated from *E. coli* [15], wheat germ [13,16,17] or Krebs cells and reticulocytes [18]. It has been

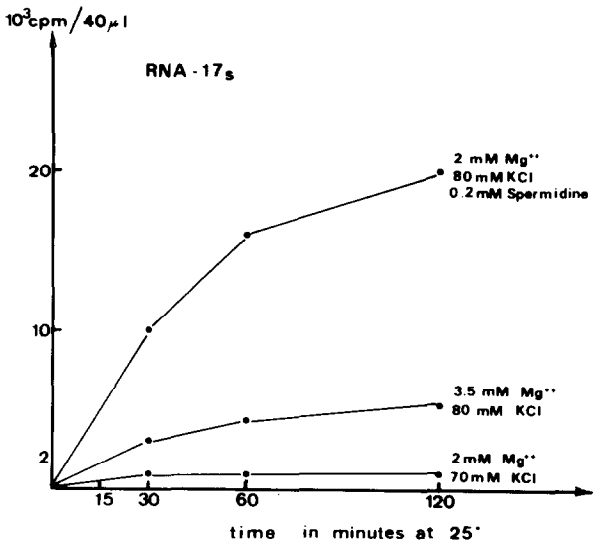

Fig.4. Stimulation of 17S RNA directed incorporation of aminoacids by spermidine. Experimental conditions are identical to that described in fig.2 where AMV total RNA is replaced by 17S RNA at a concentration of 110 µg/ml.

equally shown that the AMV 17S RNA codes for the synthesis of a protein of 35 000 daltons in wheat germ extracts [13,17] or in animal cell systems [18]. The presence of polyamines does not modify significantly the pattern of the translation products (fig.5a). In fact, the translation of 17S RNA in the wheat germ extract used and in the absence of polyamine is such a low efficiency that the 35 000 daltons product is hardly observed. In the presence of polyamines the highly stimulated translation is accompanied by the appearance of a series of polypeptide bands, especially those of lower mol. wt. This qualitative difference could result from synthesis of new peptides in the presence of polyamines as well as from an increase in the quantity of these polypeptides detected. Nevertheless, the coat protein cistron does not seem to be translated. Why the other part of 17S RNA is not translated is not yet understood.

In the presence of polyamines, 20S and 24S

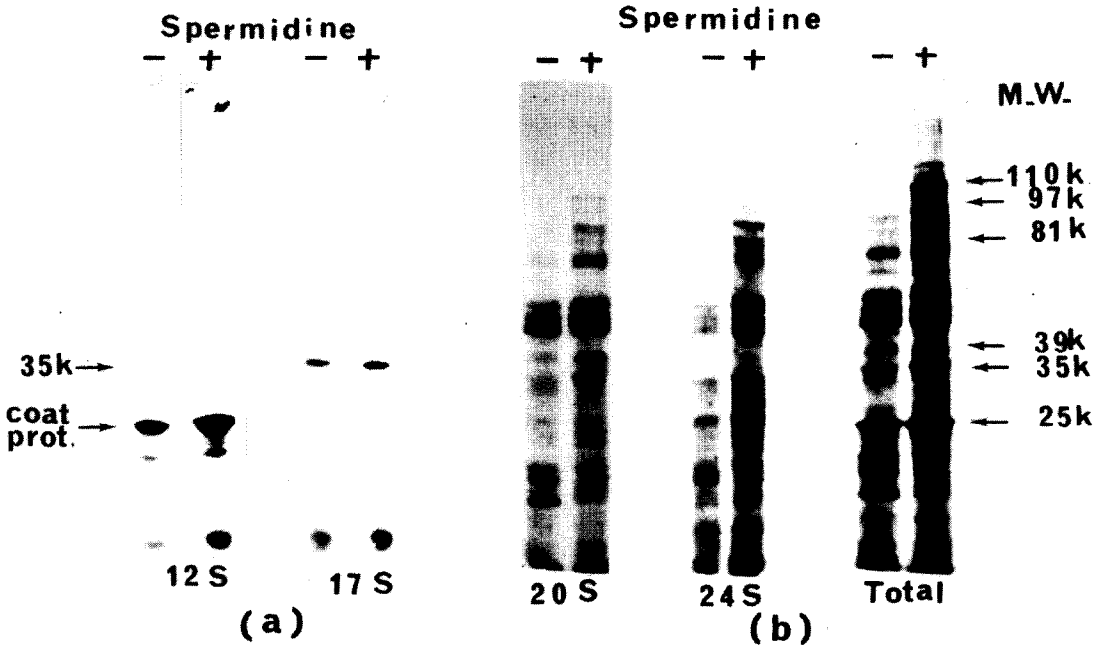

Fig.5. Electrophoretic pattern of polypeptides directed by AMV RNA in the presence or absence of spermidine. Incubation (120 min) without spermidine was performed under the conditions described in methods. Incubation with spermidine was performed at 0.2 mM spermidine and 2 mM Mg^{2+}. Aliquots of 40 µl were treated at 100°C in the presence of SDS and mercaptoethanol and layered on SDS-polyacrylamide (10%) slab gels. Electrophoresis was carried out for 5 h. Fluorography [12] was applied to detect radioactivity: (a) Film obtained with an exposure of 48 h. (b) Film obtained with an exposure of a week.

RNAs are translated into a series of polypeptides including species of mol. wt 90 000 and 110 000 respectively. These largest polypeptides are also present among the products of the translation of the total AMV RNA. In these cases, polyamines have a major effect in maximizing the sizes of the translation products (fig.5b).

4. Discussion

Polyamines have been shown to be involved in many steps of protein synthesis [19] though the mode of action is still not elucidated.

Translation of alfalfa mosaic virus RNAs in a wheat germ extract is shown to be stimulated by addition of spermine or spermidine, as has been shown for the incorporation of amino acids level directed by TMV RNA [20]. The wheat germ extract used has been prepared by a procedure involving a Sephadex G-25 gel filtration step. This filtration has been shown to result in elimination of the wheat germ polyamines (T. Hunt, personal communication). Replacement of wheat germ polyamines by spermine or spermidine may restore the initial wheat germ extract composition. However, the fact that stimulation observed can be partly obtained by a preincubation of the extract before translation without addition of these polyamines suggested that polyamines might not be a factor absolutely required for the translation of the viral RNA. The polyamine effects seem to possess several aspects. They suppress the lag phase observed during the translation, presumably due to an activation of the charging of tRNAs, and a structural modification of the viral RNA as suggested by the different behaviour of the TMV, BMV and AMV RNA in preincubation experiment. The striking effect on the 17S RNA translation might be due to a renaturation of this RNA which could be isolated in a denatured form during the separation procedure. Equally, polyamines might maximize the size of polypeptides synthesized either by preventing the premature termination of translation or by inhibiting the degradation of the products.

Acknowledgement

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