

## EFFECT OF $Mg^{2+}$ IONS ON THE IN VITRO TRANSLATION OF RED CLOVER MOTTLE VIRUS M RNA

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*Summary.* — Red clover mottle virus middle-component RNA was translated in rabbit reticulocyte lysate into two primary polypeptides with molecular weights of 95 000 and 105 000. The relative ratio of the two polypeptides synthesized was affected by  $Mg^{2+}$  concentration.

*Key words:* red clover mottle virus M RNA; *in vitro* translation; effect of  $Mg^{2+}$ ; rabbit reticulocyte lysate

The genome of red clover mottle virus (RCMV; Comovirus group) is composed of a large — B (mol. wt.  $2.4 \times 10^6$ ) and a small — M (mol. wt.  $1.4 \times 10^6$ ) plus-type, single-stranded RNA segment (Marcinka, 1983). RCMV M RNA is polyadenylated at the 3' end and a small protein (VPg) is covalently linked to its 5' end. Complete sequence of 3543 nucleotides of M RNA was determined and molecular weights of translation products (109 452 and 101 222) were calculated from the primary structure (Shanks *et al.*, 1986). Goldbach and Krijt (1982) found 102K and 96K primary translation products of RCMV M RNA by *in vitro* translation in rabbit reticulocyte lysate.

RCMV isolate TpM 25 was purified from infected pea (*Pisum sativum*) leaves (Marcinka, 1971). Viral RNA was prepared from purified virus according to Pelham (1979) and the two segments were separated by centrifugation in 10—30% sucrose density gradient in a Beckman VTi 50 rotor at 50 000 rpm for 2 hr and at 13 °C. Fractions of M RNA were collected and precipitated with ethanol. *In vitro* translation was done in a mRNA-dependent rabbit reticulocyte lysate (MDL) prepared according to Pelham and Jackson (1976). Conditions of translation were as described by Pelham (1979) except  $Mg^{2+}$  concentration. Portions of the translation mixture were adjusted to various concentrations of  $Mg^{2+}$  ions. The MDL itself contributed about 2 mmol dm<sup>-3</sup>  $Mg^{2+}$  (confirmed by flame atomic absorption spectroscopy). Translation products labelled by <sup>35</sup>S-methionine (Amersham, 30 TBq/μmol) were analysed by 8—18% gradient SDS-polyacrylamide gel electrophoresis (Laemmli, 1970).

Molecular weights of two primary translation polypeptides were determined as 95K and 105K. Detectable amounts of these polypeptides were obtained after addition of: 0.5 mmol dm<sup>-3</sup> EDTA (to obtain a final  $Mg^{2+}$  concentration of <2 mmol dm<sup>-3</sup>); no  $MgCl_2$ ; 0.5 mmol dm<sup>-3</sup>  $MgCl_2$ ; 1.0 mmol

$\text{dm}^{-3}$   $\text{MgCl}_2$  and  $1.5 \text{ mmol dm}^{-3}$   $\text{MgCl}_2$ . The polypeptides were not synthesized when the amount of EDTA or  $\text{Mg}^{2+}$  added was higher than  $0.5 \text{ mmol dm}^{-3}$  or  $1.5 \text{ mmol dm}^{-3}$ , respectively. In the presence of  $0.5 \text{ mmol dm}^{-3}$  EDTA, only small amounts of the 95K polypeptide, but no 105K polypeptide was synthesized. The relative amount of 105K polypeptide increased with increasing  $\text{Mg}^{2+}$  concentration (as compared to 95K polypeptide). On addition of  $1.5 \text{ mmol dm}^{-3}$   $\text{Mg}^{2+}$ , synthesis of 105K polypeptide was preferred, though to a low level (Fig. 1).

Many authors reported that at certain concentration of  $\text{Mg}^{2+}$  ions *in vitro* translation in rabbit reticulocyte lysate does not terminate at the stop (amber) codon (Pelham, 1978, 1979; Harbison et al., 1985). Our results suggest that the level of  $\text{Mg}^{2+}$  ions in *in vitro* translation affected both start codons of RCMV M RNA. At a total  $\text{Mg}^{2+}$  concentration higher than  $2.5 \text{ mmol dm}^{-3}$ , proteosynthesis preferentially started from the AUG codon nearer to the 5' end. When  $0.5 \text{ mmol dm}^{-3}$   $\text{Mg}^{2+}$  ions was added, both polypeptides were synthesized in equal amounts.

The available data suggest that the concentration of  $\text{Mg}^{2+}$  ions affects the termination of polypeptide chain synthesis as well as initiation at alternative start codons.

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*Explanation to Figure (Plate LXXVI):*

*Fig. 1.* Fluorogram of  $^{35}\text{S}$ -methionine-labelled polypeptides of RCMV M RNA translated *in vitro* in rabbit reticulocyte lysate and separated on 8–18% gradient SDS-polyacrylamide gel. To  $15 \mu\text{g/ml}$  M RNA was added: 2 –  $1.5 \text{ mmol dm}^{-3}$  EDTA; 3 –  $1.0 \text{ mmol dm}^{-3}$  EDTA; 4 –  $0.5 \text{ mmol dm}^{-3}$  EDTA; 5 – no  $\text{MgCl}_2$ ; 6 –  $0.5 \text{ mmol dm}^{-3}$   $\text{MgCl}_2$ ; 7 –  $1.0 \text{ mmol dm}^{-3}$   $\text{MgCl}_2$  and 8 –  $1.5 \text{ mmol dm}^{-3}$   $\text{MgCl}_2$ . Lane 1 – control without RNA.